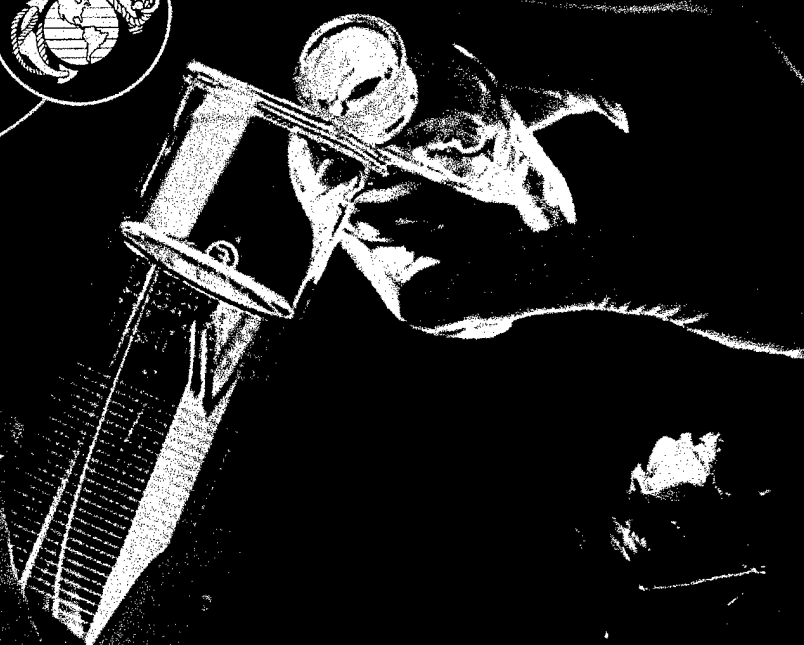


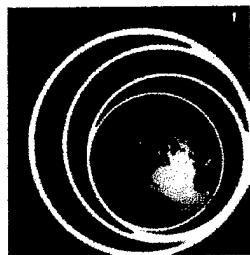
CENTER FOR BIOENVIRONMENTAL RESEARCH

at Tulane and Xavier Universities

Office of Naval Research
Annual Productivity Report: 1999-2001

ONR N00014-99-1-0763





**CENTER FOR
BIOENVIRONMENTAL RESEARCH**
at Tulane and Xavier Universities

Integrated Bioenvironmental Hazards Research Program

US Department of the Navy
Office of Naval Research



Productivity Report, 1999 - 2001
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ANNUAL PRODUCTIVITY REPORT
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Center for Bioenvironmental Research At Tulane and Xavier Universities
Bioenvironmental Hazards Research Program
Office of Naval Research/US Department of Defense
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ABSTRACT

Beginning in April 1999, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities has received funding from the Office of Naval Research to continue its Bioenvironmental Hazards Research Program (BHRP). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. The BHRP program also includes mechanisms for the effective communication of this information for resolution of Department of Defense problems and for the educational training of future scientists.

One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health, and development of biosensors for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or extending to ecological function levels. Given the CBR's and Navy's mutual interest in biosensors and small scale turbulence, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on the small scale turbulence of the model ecosystem of the Mississippi River/Gulf of Mexico and also the development of biosensors and autonomous monitoring platforms for ONR and DOD applications. As a suite of integrated modules, specific projects thus complement each other as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for DOD.

Seventeen research projects have been conducted in the two primary research modules and have resulted in significant progress related to the overall grant objectives. Assisting in the implementation of the overall project to promote the dual resolution of DOD problems and also the education of students and the general public was the continuation of four support cores: 1) environmental informatics; 2) computer operations; 3) research support; and 4) communication and education. In addition to the new knowledge developed by the research effort, the program has produced 2 useable technologies for further development, 72 publications and abstracts, 56 presentations, and supported the intellectual development of 5 postdoctoral students, 27 graduate students, 42 undergraduates, and 6 CBR SPRITE students.

This program reflects the CBR's existing research strengths and employs a set of complementary, integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences.

List of Acronyms and Abbreviations

AP-1	activation protein 1
AUV	autonomous underwater vehicles
BHRP	Bioenvironmental Hazards Research Program
CBR	Center for Bioenvironmental Research
COAMPS	Coupled Ocean Atmosphere Mesoscale Prediction Systems
COTS	Commercial off-the-shelf
DES	diethylstilbestrol
DNA	deoxyribonucleic acid
EARS	Environmental Ambient Recording Systems
EDCs	endocrine disrupting chemicals
EDTA	ethylenediaminetetraacetic acid
EEB	Ecology and Evolutionary Biology (<i>formerly EEOB</i>)
EEE	Eastern Equine Encephalitis
EPA	Environmental Protection Agency
ER	estrogen receptor
GC/MS	gas chromatography/mass spectrometry
GIS	Geographical Information System
GPS	Geographic Positioning System
LBM	Lattice-Boltzman Method
LEAG	Long-Term Estuary Assessment
MAPK	mitogen-activated protein kinase
MCB	Molecular Cell Biology
MD	Molecular Dynamics
MoA	Memorandum of Agreement
NAVOCEANO	Naval Oceanographic Office
NRL	Naval Research Laboratory
PAH	polyaromatic hydrocarbons
POC	particulate organic carbons
PPCP	pharmaceutical and personal care products

QSAR	Quantitative Structure Activity Relationships
REMUS	Remote Environmental Monitoring Underwater System
SPRITE	<u>S</u> ummer <u>P</u> ipeline <u>R</u> esearch <u>I</u> nitiative: the <u>T</u> ulane <u>E</u> xperience
TGF- β 1	transforming growth factor beta
TNF- α	tumor necrosis factor alpha
TUHSC	Tulane University Health Sciences Center
UV	ultra violet
XU	Xavier University

**Center for Bioenvironmental Research
At Tulane and Xavier Universities**

**Bioenvironmental Hazards Research Program
Office of Naval Research/US Department of Defense**

**Annual Productivity Report
N00014-99-1-0763**

I. OBJECTIVES AND SIGNIFICANCE

The Office of Naval Research (ONR) is interested in long-range science and technology research projects that offer potential for advancement and improvement of naval operations and encourages participation by Historically Black Colleges or Universities (HBCU's). This productivity report describes an integrated program of basic and applied bioenvironmental research for technology development, communication and education that supports the ONR Bioenvironmental Hazards Research Program (BHRP). The work described in this report builds upon the DOD's eight-year, integrated research program (BHRP) conducted at the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities. The CBR focuses on the holistic concept of environmental signaling from molecular to ecosystem levels, with a particular emphasis on development of biosensors and biomarkers of exposure for human and ecological systems, that addresses bioenvironmental problems relevant to the Navy and the Department of Defense (DOD). CBR research taps the basic and applied strengths of two universities that have been directed and refined over an eight-year period to reflect and, indeed, anticipate DOD environmental research interests.

A. Environmental Priorities of the Office of Naval Research

Many of the DOD environmental programs seek to understand the fate and biological effects of pollutants and contaminants resulting from military operations and training. To achieve this goal, DOD has focused on basic research to understand the biological actions of these agents, biomarkers of exposure, mechanisms of toxicity, and the use of experimental and computational modeling to assess potential health risks. The ONR focuses on "brown water" problems of particular Navy relevance (e.g., rivers, estuaries, and continental shelves). Of particular interest to the ONR's Biomolecular Science and Technology Program are biosensor/biomolecular recognition, molecular arrays, and cell-based sensors; biomaterials and process including biomineralization, biocatalysis, and bioadhesives; and biotechnologies for bioluminescence and metabolic engineering.

Central to meeting these challenges is the continued development of new knowledge, technology, and human resources through the nation's universities, where approximately half of the defense science and technology research is currently performed. The ONR is committed to strengthening the scientific capability of colleges and universities with significant enrollments from minorities underrepresented in science and engineering. Support of the nation's university-based science and education enterprise is an essential component in addressing the environmental concerns facing the DOD.

In April 1999, the Office of Naval Research awarded the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities a two-year grant of \$4.66 million in funds for the Bioenvironmental Hazards Research Program (BHRP) with a Supplemental Award of \$931 thousand in July 2000 (as modified in September 2000). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. This productivity report also includes mechanisms for the effective communication of this information for resolution of DOD problems and for the educational training of future scientists.

B. Prior Progress of the CBR Biohazards Research Program

The CBR BHRP to date has developed an integrated approach to increasing the knowledge base of actual and potential impacts on human health and ecological systems of defense-related operations, as well as the processes to restore contaminated environments. To facilitate the long-term security of the Navy, other military services, and the nation, the CBR BHRP has:

- Produced a vast suite of technologies and methods for biohazard monitoring and characterization of ecosystem, wildlife, and human health for application biohazard risk assessments for all of the DOD's branches;
- Developed one of the world's most robust programs for biosensor and biomarker technology development for real-time cost-effective monitoring of heavy metal and organic contaminants and combat-related biohazards in the air, water, and the soil;
- Facilitated a strategic partnership between academia, military, agency, and the commercial sector in the Gulf South region for a holistic long-term monitoring program for the Mississippi River, the Gulf of Mexico, and air shed to serve as a national testing laboratory for military biohazard monitoring, characterization, and communication; and
- Funded more than 100 projects over the past decade, including those through the ONR, resulting in hundreds of publications and collaborations and investments with the commercial sector.

These projects are being conducted through a wide variety of methods including *in vitro*, *in vivo*, epidemiological, modeling, field, and other laboratory studies at Tulane and Xavier Universities and associated sub contractual institutions. The significance of this research includes a greater understanding of human and ecosystem responses to environmental contamination and their ability to repair or reverse these effects; increased safety for defense workers and the general public from exposure to toxic exposures; and advanced monitoring technologies of the environment for the improvement of human and ecosystem health.

An additional benefit of CBR's BHRP is the education and research training provided to minorities. Past research activities have trained both minority faculty and students at Xavier University in scientific analytical techniques and have increased minority representation in these fields. These are techniques applicable to a variety of environmental concerns, but of particular relevance to ONR problems.

The most important result of CBR's BHRP is the development and practical application of basic knowledge and new technologies. BHRP technologies have been demonstrated through numerous environmental applications in both the defense and private sectors. Through its research, the CBR has attracted corporate investments including: \$1 million from Shell, \$75,000 from TRW, and other investments from Uniroyal and Exxon. Many of these BHRP projects have demonstrated either existing or potential commercial and civilian interest or partnerships. The useable technologies that have demonstrated the greatest commercial and civilian interest to date are summarized in **Appendix C**.

C. Current Research Efforts of CBR Biohazards Research Program

This program reflects the CBR's existing research strengths and employs a set of complementary, integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems. The integration of all the research modules has resulted in a comprehensive program of environmental research that provides the ONR with a technology package that spans research initiation to communication of environmental findings to appropriate target audiences. There are few, if any, academic organizations with this capability.

One module, Environmental Signals and Sensors, utilizes basic research on how chemical signals on molecular, cellular, and organismal levels can be utilized for assessments of human, wildlife, and plant health, and development of biosensors for assessments of toxicity and risk. Areas of focus, including human and ecological health, integrate research themes in this module by extending environmental signaling to human health endpoints at individual and population levels, or extending to ecological function levels. Given the CBR's and Navy's mutual interest in biosensors and small scale turbulence, a second module, Ecosystem Monitoring and Assessment, has placed special research emphasis on the small scale turbulence of the model ecosystem of the Mississippi River/Gulf of Mexico and also the development of biosensors and autonomous monitoring platforms for ONR and DOD applications. Cores that provide research support include computer operations, environmental informatics, and communication and education to promote the dual resolution of DOD problems and also the education of students and the general public. As a suite of integrated modules, specific projects thus have complemented each other as part of a holistic BHRP to aid in effective and comprehensive environmental assessments for the DOD.

II. CBR Capacity

A. Introduction to the CBR

The mission of the Center for Bioenvironmental Research (CBR) *is to conduct and coordinate research and teaching to enhance global understanding of environmental issues and provide solutions through innovative communication and technology.*

Founded in 1989, the CBR is an innovative, well-established and effective partnership between a Historically Black College or University (HBCU) and a major research university that encourages scientists from multiple disciplines to work together to investigate and resolve environmental problems.

Under the leadership of Dr. John McLachlan, Weatherhead Distinguished Professor in Environmental Studies at Tulane University and an internationally recognized environmental scientist and administrator, the CBR has earned a reputation for its scientific research into the environmental problems of Louisiana. In extending its spheres of influence to the national and global problems of the environment, the CBR has brought a unique focus that reflects a community-based perspective in conjunction with scientific rigor.

CBR programs are organized around five themes:

Partnerships: The CBR integrates faculty and students from Tulane's Schools of Engineering, Liberal Arts and Sciences, Medicine and Public Health and Tropical Medicine and Xavier's Colleges of Arts and Sciences and Pharmacy in innovative ways to optimize interdisciplinary teaching, learning and research.

Human and Ecosystem Health: Integrating diverse disciplines, the CBR has developed a holistic research program focusing on the effects of environmental hormones on humans and ecosystems through the processes of environmental signaling by natural and synthetic hormones and contaminants that mimic those substances.

Water: The CBR has co-evolved its programs on Environmental Signaling and Aquatic Ecosystem Research to create effective connections. Research efforts employ laboratory and field-scale approaches to look at physiochemical, biological, and ecological impacts in the Mississippi River, Gulf of Mexico, and other aquatic and atmospheric ecosystems.

Communication & Technology: The CBR provides research-based knowledge on the origins, interactions and fate of natural and synthetic chemicals in living systems using informatics capacity particularly its data management and GIS teaching lab. Through its web-based information programs and networked digital technologies, the CBR makes complex issues understandable and provides a forum for scientific discourse.

Environmental Education: The pipeline programs provide interdisciplinary training and research opportunities for undergraduate, graduate and doctoral students and faculty. Internet-based educational programs and outreach initiatives strengthen science education on campus and in the local community and region.

B. The CBR Tulane/Xavier Partnership – a Unique Model

Tulane and Xavier Universities have developed a close working relationship in the past twelve years, aided by a common vision of academic excellence and the development of high quality educational opportunities for minorities and women. The Tulane/Xavier partnership is a well-established, effective joint venture between majority and minority universities. It is an extremely complementary relationship with respect to environmental restoration and waste management, with the Xavier University focus on education and graduate work, and the Tulane University emphasis and experience in education, research, and technology development and transfer. Optimizing the research capabilities of Tulane and the educational resources at Xavier, the reputation of both institutions enhances the

ability of the CBR to solicit resources, recruit staff and researchers, sponsor conferences, and execute successful marketing of its education and research programs.

The relationship between Tulane and Xavier Universities is the foundation of the CBR and serves as a working model for all its collaborations. Since the CBR integrates academic structures, it has the freedom to advance teaching and research by creating flexible working groups to address specific needs and problems. Administrators and researchers team up with government, private, academic, and community individuals and agencies to make use of the best intellectual and technological resources. The partnerships catalyzed by the CBR exist at local, regional, and international levels resulting in community-based solutions to environmental health problems.

1. Tulane Capabilities

Tulane University has established itself as a powerful engine of economic development for New Orleans and Louisiana. Beyond that, in its 160-year commitment to education, Tulane has developed itself as a good citizen that provides the spark of creativity and knowledge that attracts and nurtures intellectual talent while directing its resources to the needs of the community. Tulane is the largest private employer in Orleans Parish and ranks 12th in the State of Louisiana.

Since its inception Tulane has grown into one of the nation's premier institutions of higher learning, known widely for both its undergraduate teaching and cutting-edge research. Those achievements are reflected in rankings by national periodicals such as *US News and World Report* that in 2001 ranked Tulane 45th overall among all national universities, and 24th in terms of best value. Tulane University ranks among the top 10 private universities in technology transfer and among the top 25 in the amount of federal research funding.

The University enrolls a diverse student body of 12,000 students from all 50 states and nearly 100 foreign countries in its undergraduate, graduate and professional schools. The University ranks in the top 10 in Environmental Law Studies, the top 15 in Public Health and International Law, and the top 25 in Biomedical Engineering. Tulane is recognized as both a member of the Association of American Universities and a Carnegie-1 research university, one of the few in the South.

2. Xavier Capabilities

Xavier University of Louisiana is the nation's only institution of higher education that is historically African American and Catholic. The ultimate purpose of the University is the creation of a more just and humane society. To this end, Xavier prepares its students to assume roles of leadership and service in society. This preparation takes place in a pluralistic teaching and learning environment that incorporates all relevant educational means, including research and community service.

Xavier University has grown in enrollment in the past 10 years with a current enrollment of nearly 4,000 students. Nearly half of the Xavier students are from Louisiana, while the rest come from 39 other states, the District of Columbia, the Virgin Islands, and 27 countries. The student body is predominantly African American (nearly 90%), but the

university is open to all. More than half of its students currently major in the natural or health sciences.

The preeminence of Xavier in training undergraduates in science is reflected in its first-place ranking in the placement of African-American graduates in medical schools for the past 10 years. In addition, Xavier is first nationally in the number of African American students earning undergraduate degrees in both the life sciences and the physical sciences. Over 40% of all recent graduates enroll in professional and graduate schools. The National Science Foundation has designated Xavier University as a "Model Institution for Excellence". The Southern Association of Colleges and Schools accredit Xavier University.

C. CBR Support Cores

The state of the art CBR facility at Tulane Health Sciences Center contains sophisticated research equipment including exposure chambers for respiratory disease studies and a Geographical Information Systems (GIS) lab. The facility is electronically networked and physically connected to Tulane Medical School and University Hospital, thus providing easy access between laboratories and clinics. The CBR facility contains state-of-the-art research equipment including a microarray core facility, a fluorescent activated cell sorter, and a high performance gas-liquid chromatograph/dual mass spectrometer. In terms of molecular biologic capabilities, the labs also feature analytical devices, and cellular and molecular biology equipment, tissue-culture facilities, and various containment and decontamination hoods and devices, so that radiation research, protein analysis, PCR, RNA and DNA analyses and immunohistochemical and in situ hybridization procedures are routinely conducted in the course of experimentation.

The CBR also has research space on the Tulane University uptown campus. The CBR Uptown contains an additional GIS lab and many equipment cores and lab space for studying neuroscience, molecular biology, and analytical chemistry. The CBR faculty located at Xavier University utilizes modern research and office space in the new seven-module environmental toxicology research center.

1. Research Support

The CBR has a first-rate management team in place with administrative capabilities to develop, implement, facilitate, track, and monitor grants and contracts as well as provide programmatic direction and administrative leadership for the facility. This team includes a director, a deputy director, associate directors, program managers and coordinators, accountants and administrative secretaries. Tulane and Xavier are innovators among universities in facilitating the mechanisms that allow scientists from multiple disciplines to work together in resolving environmental problems. In addition, the organizational structure of the CBR has allowed it to qualify for large integrated, interdisciplinary grants that are beyond the scope of many other universities or research organizations, including individual Tulane and Xavier departments. The work of the CBR is strengthened by this partnership that can provide the faculty, students, and resources that are necessary to conduct the bioenvironmental hazards research projects discussed in this report.

2. Computer Operations

The CBR has established a Computer Operations Core to modernize and facilitate efficient data communication, sample tracking, QA/QC, and data dissemination. Presentation capabilities are enhanced by a state-of-the-art computer operations facility that provides graphics and electronic media-based services and lab-based microscopy with a digital camera and PC with Image Pro archiving and image analysis software.

The Center provides fast, accurate access to the results generated by high tech analytical instruments located in various Tulane and Xavier scientific laboratories. The SGI equipment is primarily utilized for molecular modeling and other supercomputing tasks that require symmetric multiprocessing and geometry engines for advanced 3-D rendering. Apple and PC notebooks enable the CBR staff to communicate with the department by establishing a link for Internet access, electronic mail, and data transfers while attending events away from the home base. In addition to the equipment currently maintained at satellite locations, the CBR has shared access to Tulane University distributed computing environment of high performance RISC computers. These machines enable the Tulane community to stay connected with other institutions that are part of Internet 2 at data transfer speeds in excess of 100 Mbit/sec as well as other Internet entities at 10Mbit/sec.

During the ONR project period, the Computer Operations Core established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange including networking protocols, web-based information exchange procedures, and data warehousing and safekeeping.

3. Environmental Informatics

During the ONR project period, the Environmental Informatics Core provided spatial (GIS) analysis, remote sensing, mapping, database development and online systems development in collaborative projects with BHRP researchers. Major accomplishments in collaborative research included:

- GIS analysis showing that the lower Mississippi River has been aggrading from the New Orleans metropolitan area up to Baton Rouge, and deepening down to Head of Passes, with most of this bathymetric change occurring between 1921 and 1948
- Development of an online database (<http://arbonet.caeph.tulane.edu>) for researchers to log mosquito data for arbovirus surveillance in Louisiana
- Creation and maintenance of online data on the lower Mississippi River and Gulf of Mexico (<http://mirir.tulane.edu/main.htm>)
- Map of residences of uterine fibroid patients in New Orleans, Southeastern Louisiana, and US

4. Communication and Education

Funds were allocated from the Research Support core to cover student support for research teams. In this way the CBR could assist research faculty to build and train sufficient qualified personnel to complete research and also create capacity in undergraduate students. Funds were also provided for six projects in the Communication and Education core for training of students through educational initiatives. They were: 1) an environmental First Year Experience for freshmen; 2) Environmental Studies curricula

development; 3) undergraduate projects in environmental research and design; 4) two education pipeline pilot programs; 5) a series of cooperative projects with the New Orleans District Corps of Engineers; and 6) annual international symposium of presentations, posters and workshops on environmental signaling (the e.hormone conference).

First Year Experience: In the Environmental Studies program at Tulane, the academic curriculum was expanded from the Environmental Studies Coordinate Major to include an Environmental Policy track in conjunction with the School of Public Health and Tropical Medicine. In addition, the Mississippi River Colloquium that grew out of an Environmental Studies faculty seminar became the framework of a new student living-learning community. Beginning in January 2000, a planning committee developed and implemented a residence-hall based academic "First Year Experience" for undergraduates on environmental issues, using the Mississippi River basin as a natural laboratory. Twenty-eight students enrolled in the River Village Living Learning Community in Fall 2001, and the Mississippi River Colloquium has been expanded to meet increased student demand.

Environmental Studies Curricula Development: New co-curricular programs and components, including an energy-efficient model residence-hall room, have been created to enhance understanding of environmental issues. Students in the River Village created a guide (also on the web) to outdoor resources in Louisiana and Mississippi.

Undergraduate Environmental Research and Design Projects: A major accomplishment under the direction of the Office of Environmental Affairs was the funding support for an education program that enhances environmental problem-solving skills through projects that improve the environment in and around Tulane University. Projects included a recycling system design and education curriculum that involved over 100 metropolitan elementary and high school students; a guide to Tulane University campus trees; a guide for testing local surface waters for pharmaceutical contaminants; and an environmental curriculum on recycling for elementary school students. Over 20 Tulane faculty and staff have been involved as advisors in the program.

Education Pipeline Pilot Programs: With ONR support, the CBR initiated two pilot programs as part of its undergraduate education pipeline initiative to increase the number of African American students enrolling in graduate science programs. The first pilot, Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) provided Xavier undergraduate students a graduate-level laboratory research experience and mentored introduction to graduate life. In two years, this program has become a major pipeline of minority students to Tulane graduate and health professional schools. Of the 12 students who completed these two summer programs, 4 are in Tulane graduate health science programs (MPH or MD) and 4 are pursuing doctoral degrees in the Tulane Molecular and Cellular Biology program or Xavier College of Pharmacy. Three others are enrolled in MS or PHD graduate programs elsewhere, and one student took a year off before deciding on graduate school.

A second pilot education pipeline component, a joint degree program between Xavier Chemistry/Biology Departments and Tulane Engineering, has as its intent the production of minority graduates with undergraduate degrees plus MS degrees in engineering.

Initiated in Fall 2000, three Xavier students began their fourth (undergraduate) year by co-enrolling at Xavier and Tulane Universities. A summer of research followed by a full academic (5th) year at Tulane fulfills course requirements for the master's degree as well as the undergraduate degree. These students are expected to graduate at the end of summer 2002 as planned. The team effort of Tulane and Xavier faculty in mentoring the students and collaborating on ONR research projects has helped to further the consolidation process of a formal articulation agreement instituting this joint degree program.

Cooperative Army Corps of Engineers Projects: A series of cooperative initiatives including information transfer, internships, and graduate training related to historic preservation and the Mississippi River were undertaken by Tulane University faculty in conjunction with the New Orleans District Army Corps of Engineers. These projects included development of a joint doctoral program in historic preservation; creation of internship agreements and opportunities with the Corps, National Park Service, and archival facilities; institution of a spatial data inventory for the Corps; and exploration of linkages with New Orleans Public Schools and Mississippi River Parkway Commission.

International Environmental Hormone Symposia: One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is understanding how bioenvironmental contaminants can impact the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (eg. contaminants and pollutants) and its effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. For the past three years, the annual Environmental Hormone Symposium (*e.hormone*) in October initiated and hosted by the CBR has been a national and international focal point for all those who are interested in the field of environmental signaling.

The goal of the *e.hormone* symposium series is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on endocrine disruption and contribute to the future of this emerging field. The symposium format includes scientific presentations grouped around conceptual themes. Preeminent experts in the field introduce sessions and provide historical perspective on their topic and highlight recent findings. The primary strength of this annual forum of information exchange and collegial interaction is its multidisciplinary and multinational nature. Ecologists, chemists, endocrinologists, toxicologists, zoologists, engineers, philosophers, undergraduate science professors, high school teachers, policy makers, and media from the United States, Japan, Europe, and Latin America meet yearly to analyze environmental hormone issues. Each of the past three symposia has been reported on the web, and its scholarship recognized in publications such as *Science News*. The proceedings from *e.hormone* 2000 were published as a volume in *the Annals of the New York Academy of Sciences*.

In summary, the CBR is dedicated to training students for careers in science. The CBR sponsors numerous programs to increase and enhance undergraduate, graduate, and faculty

research and training opportunities at Tulane and Xavier Universities. A primary CBR goal is to encourage and enhance minority participation and representation in the sciences. The Communication and Education core reflects a collection of enriching environmental education programs that promote awareness of pertinent issues, offer interactive encounters between young and veteran scientists, and provide career-building research experiences in bioenvironmental, biomedical, and environmental sciences.

III. OVERVIEW OF RESEARCH

A. Introduction

Research associated with environmental problems of importance to the DOD required an integrated approach from fundamental science to communication of research results. This report provides a set of "integrated research modules" which will continue the CBR/ONR BHRP partnership and serve as a model for other DOD research along environmental lines. These research projects refine our existing effort to elucidate fundamental biological processes and systems that are affected by contaminants and the impacts of these changes. These assessments are a step toward modifying or eliminating the most potent effects and restoring balance into the ecosystems. One of the benefits of this modular approach is the applicability of these approaches to environmental problems using research themes or technologies that are common across platforms. An additional benefit of these reports is the provision of a mechanism for the bi-directional flow of initiatives and insights from the Navy to and from academia, while providing for the education of future scientists.

In order to select the best investigator-initiated research ideas for the Bioenvironmental Hazards Research Program (BHRP), the CBR held in spring 1999 an internal competition for two-year research projects from Tulane and Xavier faculty and research teams. Scientific merit was the criterion utilized in the selection process that was conducted by an interdisciplinary external review panel from academic experts from other universities and DoD branches including the Navy, Army, and Air Force. The formal review session consisted of 3 steps: triage, merit review, and ranking. To ensure that project selections were made that best represented DOD bioenvironmental program objectives, the external review panel evaluated all proposed projects utilizing the following criteria: technical merit, relevance to program objectives, qualifications of researchers, availability of required resources to conduct the proposed research, and appropriateness of the proposed budget and duration. Allocations by CBR Directors in July 1999 were based on the rankings determined by the panelists during the review process.

In the area of Environmental Signals and Sensors, eight projects were funded. Of these eight, two were joint Xavier/Tulane efforts, led by the Tulane investigator. In the area of Ecosystem Monitoring and Assessment, eight projects were funded. Of these eight projects, a Xavier investigator led one, and one was a joint Tulane/Xavier effort. In the Communication and Education area, seven projects were funded. One was a joint Tulane/Xavier effort, and one was a sub-contract with the US Army Corps of Engineers. A total of twenty-three (23) projects were funded under the CBR BHRP award of April 1999.

B. Environmental Signals and Sensors

A principal goal of our ongoing research is to examine the actions of DOD-relevant contaminants/pollutants (e.g., organo-chlorine compounds, PAH's, and heavy metals) on important cell targets. Long-term goals of the research in this module are to identify suitable biological markers that will serve as early indicators of toxicant exposure in humans and wildlife and potentially of the overall health of the ecosystem, thereby linking with the other research module in this project. The research addressing this theme will elucidate a scientific basis to develop rational biologically based risk assessment models.

In the area of **Environmental Signals and Sensors**, eight (8) projects were funded.

- **Dr. Thomas Bishop**, Assistant Professor of Environmental Health Sciences, with Co-Investigator Dr. Thomas Wiese, Assistant Professor of Environmental Health Sciences, Tulane and Xavier Universities, tested homological modeling using multiple molecular dynamics simulations and docking studies as a new method of analysis of ligand-receptor interactions (*"Quantitative Structure Activity Relationships from Molecular Dynamics: a Computational Method of Identifying Environmental Estrogens"*).
- **Dr. Thomas Bishop**, Assistant Professor of Environmental Health Sciences, Tulane University, with Co-Investigator Mr. Oleksandr Zhmudsky, MS, CBR Research Scientist, developed mathematical and computational models of signaling through DNA and chromatin (*"Mathematical Models of Signaling"*).
- **Dr. Diane Blake**, Associate Professor of Ophthalmology, Tulane University, with Co-Investigators Dr. George Flowers, Associate Professor of Geology, Tulane University, and Dr. Robert Blake, Professor of Basic Pharmaceutical Sciences, Xavier University, used a set of high-affinity, highly selective binding reagents to develop an immunosensor for EDTA that can operate in an autonomous underwater vehicle (*"Antibody-Based Biosensors for Autonomous Underwater Vehicles"*).
- **Dr. Matthew Burow**, Research Assistant Professor of Pharmacology, with Co-Investigator Dr. John McLachlan, Professor of Pharmacology, Tulane University, identified a basic mechanism by which selected environmental agents subvert the estrogen and cell survival signaling pathways, thereby leading to potential developmental defects and/or disease states (*"Effects of Estrogens and Endocrine Disrupters on Suppression of Apoptosis in Normal and Neoplastic Breast Epithelial Cells"*).
- **Dr. Mitchell Friedman**, Professor of Pulmonary Diseases, with Co-Investigators Dr. Arnold Brody, Professor of Pathology, and Dr. Joseph Lasky, Associate Professor of Pulmonary Diseases, Tulane University, studied how signals on cellular and molecular levels can be utilized for assessment of human health and development of biosensors for assessments of toxicity and risk in the lung (*"Airway Epithelial Signaling as a Mechanism of Lung Injury to Inhaled Toxicant"*).
- **Dr. Scott Michael**, Assistant Professor of Tropical Medicine, Tulane University, determined from this research project that frozen sperm from an extinct species are able to direct development of enucleated eggs from a closely related extant species (*"Ecological Remediation: Resurrection of an Extinct Species of Frog from Puerto Rico"*).

- **Dr. Dawn Wesson**, Associate Professor of Tropical Medicine, with Mr. Richard Campanella, CBR Environmental Analyst/GIS Specialist, Tulane University, characterized the habitat preferences, spatial patterns, and temporal trends in mosquito species at sites in the semi-rural North Shore of Lake Pontchartrain. This project targeted, in particular, *Culiseta melanura*, a species which has long been implicated as a maintenance vector of the Eastern Equine Encephalitis cycle throughout the United States ("*Field Experiment to Characterize Habitat Preferences of Key Mosquito Species on the North Shore of Lake Pontchartrain, Louisiana*").
- **Dr. Valerie Wilson**, Clinical Associate Professor of Environmental Health Sciences, Tulane University, identified and described environmental and other risk factors for uterine fibroids in a population of New Orleans women ("*Human Health Applications*").

C. Ecosystem Monitoring and Assessment

The Navy requires a fundamental understanding of fate, transport and transformation effects of contaminants in estuarine and near-shore environments. Since DOD operations can frequently result in the release of a variety of perturbations in a region, a holistic assessment of potential biohazard impacts must include ecosystem-level analyses. To conduct an ecosystem health assessment like this, it is necessary to understand the health of humans and wildlife present in a particular region, the interactions of these species with each other on a ecological population and community level, and the interactions of the physicochemical environment with that of the biota.

In the area of **Ecosystem Monitoring and Assessment** eight (8) projects were funded.

- **Dr. Glen Boyd**, Assistant Professor of Civil and Environmental Engineering, with Co-Investigator, Dr. Siddhartha Mitra, Senior Research Scientist, Tulane University, developed a standard methodology for the efficient extraction of several PPCPs from the Mississippi River and coastal Louisiana particulate that can be used to develop and test hypotheses related to the efficacy of treatment processes for the removal of PPCP and EDC contaminants from water resources and wastewater ("*Extraction and Detection of Selected Pharmaceuticals and Personal Care Products (PPCPs) in Coastal Areas of Southeastern Louisiana*").
- **Dr. Bernard Coakley**, Assistant Professor of Geology, and Dr. Mead Allison, Assistant Professor of Geology, with Co-Investigator Mr. Richard Campanella, CBR Environmental Analyst/GIS Specialist, Tulane University, mapped, measured, and analyzed bathymetric changes of the lower 200 miles of the Mississippi River with resultant data indicating that from 1919 to 1992, channel changes from New Orleans to Baton Rouge varied to a slightly greater degree than has occurred in the lower 100 miles ("*Historical Bathymetry of the Lower Mississippi River, 1915-1992: Evolution of a Controlled River*").
- **Dr. J. Fernando Figueroa**, Associate Professor of Mechanical Engineering, Tulane University, conducted research to develop a generic methodology to embed

"intelligence" into sensor models ("*Generic Model to Embed Intelligence in Environmental Sensors*").

- **Dr. Chao-Jun Li**, Professor of Chemistry, with Co-Investigator, Dr. Russell Schmehl, Professor of Chemistry, Tulane University, studied the sensing mechanism of self-assembling induced enhancement and concluded that self-assembling is the determining factor that governs the sensing properties of the sensors ("*Developing Chemosensors Based on Self-Assembling Induced Fluorescence Enhancement*").
- **Dr. Brent McKee**, Professor of Geology, Tulane University, with Co-Investigators, Dr. Tom Bianchi, Associate Professor of Ecology and Evolutionary Biology, Tulane University, Drs. Mike Dagg and Rodney Powell, Louisiana Universities Marine Consortium, and Dr. Richard Miller, Chief Scientist, NASA Stennis Space Center, used remote sensing, biological and geochemical techniques to determine the major pathways of carbon in the Mississippi River ("*River-Ocean Interactions (Phase I): The Processing and Fates of Nutrients and Organic Carbon from the Mississippi River*").
- **Dr. Douglas Meffert**, Clinical Associate Professor of Environmental Health Sciences, Tulane University, pursued integration and technology co-development links to deploy biosensors in remote environments, specifically through autonomous/unmanned underwater vehicles ("*Autonomous Monitoring and Visualization Technology Development for Aquatic Environments*").
- **Dr. Efstathios Michaelides**, Professor of Mechanical Engineering, Tulane University, with Co-Investigators Dr. Laura Steinberg, Assistant Professor of Civil and Environmental Engineering, Tulane University, and Dr. Elia Eschenazi, Associate Professor of Physics and Engineering, Xavier University, studied the sedimentation and resuspension characteristics of single particles and flocs in aqueous solutions ("*Sedimentation and Resuspension Studies for the Mississippi River and Louisiana Coastal Environments*").
- **Dr. J. Yao**, Department of Chemistry, Xavier University, used kinetic modeling in soot formation in studying the suppression of PAH and POC in the context of the chemical transformations and their products ("*Emission Monitoring/Analysis of Diesel Fuels in a Shock Tube*").

IV. SUMMARY ACCOMPLISHMENTS

A. Overview

Accomplishments on this grant can be documented in three major areas:

1. **Research Publications, Abstracts and Presentations** – that document progress made in the research through publication in a number of peer-reviewed venues available to the general scientific community;
2. **Development of Useable Technologies** – which have direct benefit on furthering the mission-related scientific interests of ONR; and
3. **Intellectual Development** – that extends the ability of the grantee and ONR by developing the next generation of scientists.

For investigative research reports, see **Appendix A**.

Research Publications, Abstracts and Presentations:

Research from this project period resulted in the following publications in scientific journals and conference reports:

- Sixty (60) publications in research journals and in conference reports such as *Journal of Medicinal Chemistry, Journal of Biological Chemistry, American Journal of Pathology, Journal of Environmental Pathology, American Journal of Respiratory Cell and Molecular Biology, Journal of Comparative Physiology, Chemical Communications, Journal of Geophysical Research, Journal of Plankton Research, Geochimica Comochimica Acta, Journal of Hydraulic Engineering* to name a few.
- Twelve (12) published abstracts in meeting and symposium reports.
- Fifty six (56) presentations at major scientific conferences across the country and internationally including, but not limited to, the annual meetings of the Louisiana Mosquito Control Association, American Water Works Association, Institute of Electrical & Electronics Engineers, International Mechanical Engineering Congress & Exposition, and American Society of Limnology & Oceanography.

A complete listing of the publications, abstracts and presentation made by investigators on this project can be found in **Appendix B**.

Development of Useable Technologies:

Research results from this project have generated two potentially useable technologies.

One technology that is part of the Environmental Signals and Sensors area is:

- Immunosensor for AUV Deployment. This antibody-based biosensor will automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy. A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters. A provisional patent application entitle "Recombinant antibodies that bind to metal-chelate complexes" was filed in March 2001. Partners in this effort are Sapidyne Instruments and the Naval Research Laboratory (Dr. Fran Ligler) in Washington, DC.

The second technology that is part of the Ecosystem Monitoring and Assessment area is:

- Integrated Autonomous Immunosensor & Autonomous Underwater Vehicle System. This system will enhance real-time biosensor deployment for environmental compliance and ultimately biologic warfare detection. Coordinated research discussions are underway with COTS Technology, LLC; Woods Hole Oceanographic Institute (MA); and the US Naval Oceanographic Office (MS). Patents will be applied for by the partners.

Further details on the potential technology products can be found in **Appendix C**.

Intellectual Development:

The research effort provided for the intellectual development of the faculty who participated in the project. In the process of conducting their research, investigators collaborated with new and existing partners in research, and at times formed unique consortia and research teams. Several of the investigators worked across departmental boundaries and in a few instances, faculty members from each component university formed a Tulane/Xavier research team to undertake a project.

The project has supported the research work of:

- 5 postdoctoral students assisting in the conduct of the research of their primary investigator.
- 27 graduate students, including three who were participants in a Tulane/Xavier collaborative mentoring program (3+2= 2) leading to a master's degree in Engineering.
- 42 undergraduate students, including 5 Xavier undergraduates (4 worked with a Xavier investigator, 1 worked with a Tulane advisor), 36 Tulane undergraduates and 1 Loyola undergraduate who worked on projects with Tulane advisors. These undergraduates conducted research with a variety of investigators and thus were exposed to a variety of aspects of the overall project.
- 6 SPRITE (undergraduate) students who were chosen out of an applicant pool to follow a mentored graduate-level laboratory research experience and gave a scientific presentation of their research project results to mentors, faculty, and peers at the conclusion of the summer program.

A complete listing of the student names and principal investigators/advisors can be reviewed in **Appendix D**.

B. Environmental Signals and Sensors

- Twelve separate 1.6ns simulations using six different conformations of the ER-LBD with DES bound and with Estradiol bound were conducted to determine the stability of the ligand-receptor complex.
- Each of the ten different molecules to be docked to the receptor was modeled using three different levels of molecular modeling to determine the validity of the chosen molecular mechanics parameters.
- Twelve different mutant structures of the estrogen receptor ligand binding domain were modeled to assess the properties of the ligand receptor interactions.
- Linear analysis enabled the identification of four different types of mechanical disturbances that can propagate through an elastic rod and determination of the speed of propagation of these types of disturbances based only on the elastic properties of the fiber.
- Numerical simulations have enabled the determination of the limits of the linear theory; in particular that linear theory is only applicable for the case of unbent DNA.

- The simple model of folding suggested that the elastic properties of extended chromatin could be deduced by a simple helical folding of DNA while the properties of condensed chromatin cannot be directly related to the so-called solenoid model based only on the elastic properties of DNA.
- A new method for producing large quantities of monoclonal antibody in a tissue bioreactor has been developed.
- The genetic stability of several different hybridomas under culture in a tissue bioreactor has been determined.
- Three full-size mock-ups of the submersible immunosensor were developed with one in preparation for installation of optical components.
- Software and plumbing modifications that allow for the testing of fluidics design of the AUV-based sensor on a research grade KinExA 3000 have been installed.
- A prototype assay for EDTA, the first analyte to be developed for this instrument, has been established on the KinExA 3000 research instrument.
- An antibody specific for chelated complexes of Pb (II) has been expressed as a recombinant protein.
- A role for MAPK signaling in conjunction with Bcl-2 expression in estrogen mediated cell survival signaling in breast carcinoma cells was identified.
- A role for JNK and p38 MAPKs in signaling by flavonoid phytochemicals in the regulation of ER-mediated gene expression and proliferation of breast carcinoma cells was identified.
- A role for organochlorine pesticides and flavonoid phytochemicals signaling to AP-1 via ER-independent mechanisms was identified.
- The most important component of the in-vivo studies is the demonstration that there is a significant role of TGF- β_1 in the pathogenesis of interstitial pulmonary fibrosis, secondary to inhaled toxicants.
- Research showed that TGF- β_1 is diminished in the lungs of mice that develop a reduced disease response and that transient re-constitution of TGF- β_1 expression returned a fully developed fibroproliferative disease process to the TNF- α RKO mice.
- This research further implies that studies looking at mechanisms of injury for inhaled toxicant such as silica should not target one signaling molecule, but rather a signaling pathway.
- Artificial induction of ovulation for the production of large numbers of *E. coqui* eggs was achieved.
- Nuclear decondensation from long-term frozen sperm with the in vitro fertilization and generation of viable frogs was demonstrated.

- A detailed dataset of mosquito species and characteristics, along with corresponding ecological/geographical/climatic data, at seven sites was collected and is being analyzed.
- A grid of 15 points over a quarter-mile area was designed, selected, and established with setting up of data loggers, traps, and rain gauges to assemble data.
- Data studies of Charity Hospital and a metropolitan physician's medical records indicated that New Orleans women with uterine fibroids have a demographic and health profile similar to that of women in other cities.
- Research data indicate that a specific decline in the age of menarche in younger women with fibroids (less than 35) may implicate a developmental or environmental factor (yet to be defined) in the condition.
- Research data indicate that BMI (a measure of obesity) may be a predictor of predictor of multiple uterine fibroids.

C. Ecosystem Monitoring and Assessment

- Standard operating procedures for extraction, quantification, and monitoring of 11 PPCPs and EDCs in Mississippi River and Lake Pontchartrain waters and sediments were developed and documented.
- A detailed procedure for testing the analytical recovery of a water sample spiked with PPCPs and EDCs was developed and documented for use in environmental process-oriented research related to contaminants in aqueous environments.
- Georectified GIS database on the bathymetry and banklines of the lower Mississippi River for seven dates spanning the 20th century was created.
- Preliminary data analysis revealed trends of aggradation above New Orleans and erosion below the city.
- A method was developed to turn any sensor into an intelligent entity capable of interpreting data as a sequence of qualitative behaviors.
- Sensor fusion strategies were developed to take advantage of the rich qualitative information provided by the intelligent sensor models.
- X-ray crystal structures of the sensor molecules and lead ions of lead, cadmium, and mercury were obtained.
- The lifetime of the excited states of the sensor molecules in the free form and in the bonded form were determined.
- Sensors for lead, mercury, cadmium, and copper were developed.
- The major pathways for terrestrial carbon from the Mississippi River have been determined for two seasonal time frames in 2000 (Spring-high flow; Fall-low flow).
- Data was collected under strict conditions of coordination so that a major portion of the carbon pathway can be understood.

- The REMUS vehicle being developed by Woods Hole Oceanographic Institute was chosen as a platform for one of the CBR antibody-based biosensors of an autonomous/ unmanned underwater vehicle.
- Partnerships facilitated to promote joint, coordinated research on biosensor coupling with autonomous underwater vehicle navigation enhancement and intelligent programming.
- A code based on the LBM to study the sedimentation and re-suspension behavior of single particles and groups of particles was completed.
- Hydrodynamic lift forces exerted by a suspension of particles on a stationary particle are sufficient to cause the re-suspension of the latter without any inter-particle collisions.
- Sedimentary particles strongly interact and form dynamic clusters.
- Characteristics of the sedimentation of the clusters are totally different from those of single particles.
- The pyrolysis of organic compounds have been studied in order to find a theoretical reaction process for the pyrolysis of N-hexadecane.
- In developing an experimental model, a number of free radical reactions were approximated by a molecular model consisting of primary and secondary reactions.

APPENDIX A.

INVESTIGATOR RESEARCH REPORTS

RESEARCH CORES

Computer Operations Core

Principal Investigator: John Vassilopoulos
Associate Director
Computer Operations
Center for Bioenvironmental Research
At Tulane and Xavier Universities

Reporting Period: May 1999 – April 2001

Primary Objectives of Research Activities

To provide end-user technical direction for data warehousing, networking, securing storage for project-related data, and coordinate with research support efforts for all CBR core projects.

Progress Made to Achieve these Objectives

- Hardware and software acquisitions.
- Implementation of networking protocols.
- Web-based information exchange.
- Data warehousing and safekeeping.

Major Accomplishments

- Established and maintained the IT infrastructure necessary to accommodate all project requirements for analysis and information exchange.

Publications, Manuscripts, Abstracts

N/A

Presentations

N/A

Intellectual Development

N/A

Useable Technologies

N/A

Environmental Informatics Core

Principal Investigator: John McLachlan, Ph.D.
Weatherhead Distinguished Professor and Director
Center for Bioenvironmental Research
at Tulane and Xavier Universities

Co-Investigator(s): Douglas Meffert, Ph.D.
Clinical Associate Professor and Associate Director for Planning

Richard Campanella, MS
Assistant Director for Environmental Analysis

Stephanie Smith, MS
Online Database Manager and Systems Administrator
Center for Bioenvironmental Research
at Tulane and Xavier Universities.

Reporting Period: May 1999 – April 2001

Primary Objectives of Research Activities

The Environmental Informatics Laboratory's objectives are to provide spatial (GIS) analysis, remote sensing, mapping, database development, and online systems development by collaborating with researchers from other cores in the Bioenvironmental Hazards Research Program. This collaborative research adds value to the researchers' investigations by contributing spatial analyses, cartographic depictions, online data access, and other geography-oriented perspectives to their science.

Progress Made to Achieve these Objectives

Our collaborative research during this reporting period has been concentrated in the following areas within the Bioenvironmental Hazards Research Program. Under **Repeat Acoustic Imaging of the Mississippi River Bed**, we have been collaborating with Dr. Bernard Coakley and Dr. Meade Allison of the Tulane Department of Geology in researching and quantifying bathymetric change in the lower Mississippi River. Under **Pest Importance and Relative Distributions of Potential Virus Vectors**, we have been working with Dr. Dawn Wesson in the Department of Tropical Medicine in designing, executing, and analyzing the results of a large-scale field experiment to characterize habitat preferences of mosquitoes that are potential virus vectors. Additionally, we have provided support in developing an online database, Arbonet in which researchers log mosquito data for the purpose of arbovirus surveillance in Louisiana. Under **Ecosystem Health Applications-Endocrine-Disrupting Chemicals/Pharmaceutically Active Compounds**, we have collaborated with Dr. Glenn Boyd in storing results of water samples tested for pharmaceutical contamination in a GIS database and creating a series of maps. Additionally, under **Ecosystem Health Applications**, we have developed an online distributed GIS database to facilitate research on the lower Mississippi River and Gulf of Mexico, and prepared a series of cartographic materials regarding the National Center for the Mississippi River. Under **Human Health Applications**, we have collaborated with Dr. Valerie Wilson in mapping occurrences of

uterine fibroids in New Orleans and the nation, overlaying them on various demographic and socioeconomic data. Finally, under **Human Health Applications**, the Environmental Informatics Lab prepared and delivered biweekly GIS educational seminars to the campus community at Tulane and Xavier Universities throughout Fall 200 and Spring 2001, reaching over 100 students, professors, and staff.

Major Accomplishments

- **Repeat Acoustic Imaging of the Mississippi River Bed: Bathymetric Change in the Lower Mississippi River**

- Gathered, verified, rectified, and mapped GIS data of bathymetric points and banklines for seven dates (1921-1992) of the 200 miles of the lower Mississippi River;
- Rectified the varying geodetic parameters of these datasets by researching the historical vertical datums in the lower Mississippi Valley and applying adjustment formulae;
- Differenced the various years to map bathymetric change at the one-mile level;
- According to this analysis, the lower Mississippi River has been aggrading from the New Orleans metropolitan area up to Baton Rouge and deepening from New Orleans down to Head of Passes, at a rate of about one foot per 10 river miles, with most of this bathymetric change occurring in the 1921-1948 time period;
- Our current research is addressing the cause of this interesting pattern.

- **Pest Importance and Relative Distributions of Potential Virus Vectors**

- The objective of this field experiment, which we are conducting in collaboration with Dr. Dawn Wesson, is to characterize the habitat preferences, spatial patterns, and temporal trends in mosquito species which are potential virus vectors, at an array of sites near New Orleans, Louisiana.
- Established 7 sites (phase I of experiment, selected using remote sensing and GIS as seven distinct ecological/geographical environments) at which four mosquito traps, a data logger (recording temperature and relative humidity), and a rain gauge were set up. These sites were visited by 3-6 project participants, (23 times between February 21 and June 8, 2001). Laboratory staff then analyzed each specimen and stored the results in a database, which was then analyzed spatially.
- Established larger and more representative array of sites (phase II of experiment) totaling 15 sites and arranged as a systematic 3 x 5 grid imposed. We established this grid (using GPS and machetes) in May 2001 and began collections in mid-June 2001 at a pace of twice a week, involving 3-5 people each time. Preliminary analysis of the results show marked geographical correlations and preferences of certain key species for certain habitats.
- Provided support in developing the GIS component to an online database (<http://arboNet.caePh.tulane.edu>) for researchers to log mosquito data for arbovirus surveillance in Louisiana.

- **Ecosystem Health Applications**

- Created and maintain online distributed data to facilitate research on the lower Mississippi River and Gulf of Mexico, in support of the Long-Term Estuary Assessment Group (LEAG). This is viewable at <http://mirir.tulane.edu/main.htm>.

- Maintain GIS database and create maps for Dr. Glenn Boyd's field research for pharmaceutically active compounds in water bodies in southeastern Louisiana, including the Mississippi River.
- **Human Health Applications**
 - Mapped residences of uterine fibroid patients in New Orleans, southeastern Louisiana, and throughout the nation, in support of the Dr. Valerie Wilson's work on this disease.

Publications, Manuscripts, Abstracts

We intend to submit peer-reviewed publications on the Mississippi bathymetry project and the mosquito experiment when results are fully analyzed. The bathymetry paper is in preparation, tentatively entitled *Analysis of Bathymetric Change in the Lower Mississippi River, 1921-1992*.

Presentations

Campanella, Richard, Dr. Bernard Coakley, and Mead Allison. *Historical Bathymetry from the U.S. Army Corps of Engineers for the Lower Mississippi River, 1915-1992: Evolution of a Controlled River*. Poster Sessions at American Society for Limnology and Oceanography (February 2001), Albuquerque, New Mexico, and 17th Annual Louisiana Remote Sensing & Geographic Information Systems Workshop (April 2001), Baton Rouge, Louisiana.

Campanella, Richard, Ian Sutherland, Bryan Shelby, Dr. Dawn Wesson. *Spatial Patterns of Aedes aegypti, Aedes albopictus, and Aedes triseriatus in Greater New Orleans Region, Summer 2000*. Oral Presentation, Louisiana Mosquito Control Association Annual Conference (November 2000), New Orleans, Louisiana.

Smith, Stephanie, and Richard Campanella. *Comparison of ESRI ArcView 3.2 and Intergraph GeoMedia 4.0 on a Typical GIS Analysis Problem*. GeoSpatial World 2001 (June 2001), Atlanta, Georgia.

Smith, Stephanie, and Richard Campanella. *Variations in a Typical Spatial Analysis problem from Two leading Desktop Applications*. 17th Annual Louisiana Remote Sensing & Geographic Information Systems Workshop (April 2001), Baton Rouge, Louisiana.

Additional presentations have been made since April 2001.

Intellectual Development

1. **Student Name(s):** Jasmine Hills
2. **Funding Period:** October 2000 to May 2001 (part time)
3. **Duties and Responsibilities:** One of Ms. Hills responsibilities included digitizing historic bathymetric data of the Mississippi, to be used in a future extension of this study to pre-20th century time periods.

1. **Student Name(s):** Seth Willey
2. **Funding Period:** September 2000 to December 2000 (part time)
3. **Duties and Responsibilities:** Mr. Willey helped develop GIS databases for Louisiana in support of various Environmental Informatics Lab collaboration projects. Additionally, he studied spatial relationships between industries and socioeconomic data along the Mississippi River corridor.

1. **Student Name(s):** Spencer Kramer
2. **Funding Period:** June 2000 to August 2000 (part time)
3. **Duties and Responsibilities:** Mr. Kramer processed GIS and remotely sensed data of southeastern Louisiana in support of various Environmental Informatics Lab projects. Additionally, he studied potential impacts of oil wells located near populated areas in the region.

Useable Environmental Technologies

None

Communication and Education

Principal Investigator: Valerie P. Wilson, PhD.
Deputy Director and Professor
Center for Bioenvironmental Research at
Tulane and Xavier

Co – Investigators: See Listing Below

Reporting Period: August 1999-April 2001

Primary Objectives of Research Activities

The goal of this suite of activities is to create a pipeline of programs that provide interdisciplinary training and research opportunities for undergraduate, graduate and doctoral students and faculty. Internet-based educational programs and outreach initiatives strengthen science education on campus and in the local community and region. These programs are enhanced by the communication of BHRP research results in forums created by and for scientists.

In addition to funds allocated within research projects to support student stipends to build research teams, specialized program were developed to create an infrastructure for the conduct of science and grow an intellectual capacity in this scientific area. Six projects in the Communication and Education core provided for training of students through educational initiatives. They were:

- 1) **An environmental First Year Experience** for freshmen;
- 2) **Environmental Studies** curricula development;
- 3) **Undergraduate projects** in environmental research and design;
- 4) **SPRITE**, a summer “research pipeline” in molecular biology for Xavier undergraduates;
- 5) **A Tulane/Xavier five-year joint bachelor/ master's engineering program**); and,
- 6) **A Ph.D. Program in History and Historic Preservation** around the Mississippi River was facilitated.
- 7) **International Environmental Hormone Symposia.**

In addition to direct dissemination of research and educational initiative through traditional channels the education and communication core also provided partial support for a unique international symposia of presentations, posters and workshops on environmental signaling called e.hormone. This symposium is held every October in New Orleans, and provides a critical dissemination vehicle for the research results of the BHRP program and its investigators, students and faculty.

Progress Made Toward Achieving These Objectives

Our collected work during this research period has resulted in a number of significant accomplishments. Together these accomplishments have enhanced the learning and research enterprise of the BHRP by recruiting students from initial enrollment in Tulane and Xavier programs through transitions to graduate and professional degrees, and by providing a forum for

dissemination of scientific results in annual international forums on the topic. Individual reports from each component follow this overview. Below is a brief summary of major accomplishments from each activity.

Dr. Amy Koritz has created a living/learning experience for first year students called **The River Village**. Ms. Christine Murphey, with colleagues-Drs. Timmons Roberts and Joan Bennett-has enhanced the academic curriculum of the **Environmental Studies Program** at Tulane with new co-curricular programs and components. Dr. Elizabeth Davey of the **Office of Environmental Affairs** has instituted a competitive research and design mini-grant program with projects that improve the environment in and around Tulane University. Drs. Valerie Wilson (CBR), Dana Green-McDowelle (Xavier), with Mr. Charles Allen (CBR), expanded a pilot program for minority undergraduate summer research. The program, **Summer Pipeline Research Initiative: the Tulane Experience (SPRITE)**, has the goal of increasing the number of African-American students enrolling in doctoral science programs. Drs. Elia Eschenazi, Xavier Department of Physics, and Stathis Michaelides, Tulane School of Engineering, have initiated a second pilot education pipeline component, a **joint degree Physics BS/Engineering MS** program, for students of Tulane and Xavier Universities. It has as its intent the production of minority graduates with MS degrees in engineering in 5 years from initial matriculation. Dr. Colin MacLachlan, Tulane Professor of History, instituted a series of cooperative projects with the **New Orleans District Army Corps of Engineers** including information transfer, internships, and graduate training related to historic preservation and the Mississippi River. The Communication and Education core also provided partial support for the **international environmental hormone symposia** of presentations, posters and workshops. This symposium is held every October in New Orleans, and provides a critical dissemination vehicle for the research results of the BHRP program provided by its investigators, students and faculty.

Major Accomplishments

First Year Experience - River Village

- The River Village Living and Learning community was planned in January 2000
- Twenty-eight students enrolled in the River Village Living Learning Community in Fall 2001

Environmental Studies

- The academic curriculum was expanded from the Environmental Studies Coordinate Major to include majors in Environmental Studies and Environmental Policy track in conjunction with the School of Public Health and Tropical Medicine.
- The number of environmental studies majors increased to 45 in Fall 1999
- New co-curricular programs and components, including an energy-efficient model residence-hall room, have been created to enhance understanding of environmental issues

Office of Environmental Affairs

- Undergraduate Environmental Research and Design Projects: A major accomplishment under the direction of the Office of Environmental Affairs was the funding support for an education program that enhances environmental problem-

solving skills through projects that improve the environment in and around Tulane University.

- Eight individual student projects were funded. These projects included a recycling system design and education curriculum that involved over 100 metropolitan elementary and high school students; a guide to Tulane University campus trees; and a guide for testing local surface waters for pharmaceutical contaminants, and an environmental curriculum on recycling for elementary school students.
- Over 20 Tulane faculty and staff were involved as advisors in the program.

SPRITE

- The SPRITE program provided Xavier undergraduate students a graduate-level laboratory research experience and mentored introduction to graduate life.
- In two years, this program has become a major pipeline of minority students to Tulane graduate and professional schools.
- Twelve Students have completed the ten-week summer program and presented graduate-level research presentation in an Annual Research Symposium. In addition students have made presentations at local, regional and national forums and garnered research prizes for their presentations.
- Of the twelve students completing the program in the two year of support from ONR, 3 have enrolled in PhD programs, 2 others in MS graduate /MPH professional school and 5 are in medical school (two others are completing undergraduate training).

Joint Physics/Engineering Masters Degree Program

- Initiated in Fall 2000, three Xavier students began their fourth (undergraduate) year by co-enrolling at Xavier and Tulane. A summer of research followed by a full academic (5th) academic year at Tulane allows the completion of course requirements for the masters
- These students are expected to graduate at the end of summer 2002 as planned.
- The team effort of Tulane and Xavier faculty in mentoring the students and collaborating on ONR research projects has helped to further the consolidation process of a formal articulation agreement instituting this joint degree program.

New Orleans District Corps of Engineers PhD Program in History and Historic preservation

- The development of joint PhD program in history and historic preservation was completed.
- A Career Services internship agreement with Corps of Engineers was facilitated.
- A grant program for interns in historic preservation at National Landmark field sites was developed

International Environmental Hormone Symposia.

- The CBR has established a scientific forum for information exchange and collegial interaction for scientists, including ONR-funded researchers, involved in environmental signaling research that studies the impact of bioenvironmental contaminants.
- Through its three-year history, the e.hormone symposium has resulted in the creation

of an extensive global network.

- A "spin-off" e.hormone website has been created as a hub of scientific and media information connecting research colleagues throughout the year.
- The symposium has created a mentoring/networking forum for junior investigators.

Publications

See individual reports for listing of Publications

Presentations

See individual reports for listing of presentations

Intellectual Development

See individual reports for listing of the many students and faculty impacted by these programs

Useable Technologies

None specifically. However several of these model programs could/should be disseminated for regional/national implementation.

Freshman Year Experience

Principal Investigator: Amy Koritz, Ph.D.
Associate Professor
Las English
Tulane University

Reporting Period: January 2000 - April 2001

Primary Objectives

To plan and implement a residence-hall based academic program for undergraduates on environmental issues, using the Mississippi River basin as a natural laboratory; and to create an environment where students, faculty and staff could interact in non-traditional ways to foster collegiality and closer student-faculty relationships.

Progress Made to Achieve these Objectives

1. January 2000, a committee of faculty and staff convened to plan the River Village Living Learning Community, to be inaugurated in Fall 2001.
2. Summer 2000, a planning workshop of environmental studies faculty met for a one-week seminar to plan the curriculum for this program.
3. Fall 2000, marketing and application materials were developed to attract and enroll undergraduates.
4. Spring 2001, upper-class students wrote application essays and on this basis 31 were accepted for the River Village's inaugural year.
5. The Mississippi River Basin Course (ENST377/EEOB377/DSTP377) was offered as the principal introductory course for the village.
6. Faculty and staff assisted students during registration to plan their course work so that it would include relevant courses that would enhance their learning in the first semester. A roster of courses was developed and published for the students that included classes in all schools on campus offering courses on topics related to the Mississippi River.

Major Accomplishments

- 28 Tulane students enrolled in the River Village Living Learning Community in Fall 2001
- Mississippi River Basin Colloquium has been expanded (from 22 to 30) to meet increased student demand due to implementation of the River Village. A number of students had to be turned away when the maximum practical enrollment due to logistical requirements was met. Students participated in nine field trips (eleven had been scheduled but two were cancelled due to 9/11 events). All students and faculty participated in these trips. These field trips included one three day and one two day field trips, as well as a one day field trip. The other six trips were afternoon trips. One field trip was coordinated with the faculty member teaching the Environmental Sociology class. Students had presentations by 11 off-campus speakers, including a fisherman, a farmer, a representative of the Louisiana Tumor Registry, community activists, historic preservationists, members of the Corps of Engineers and the National Park Service, a lawyer, and non-Tulane faculty. Students, faculty, staff, and invited guests met four times for dinner, which included either student presentations, discussions, or presentations by invited guests.

- New co-curricular programs and components, including an energy-efficient model residence-hall room, have been created to enhance understanding of environmental issues for program participants. Students in the River Village created a guide to outdoor resources in Louisiana and adjacent parts of Mississippi. This guide, which will be on the web as well as in hard-copy format, provides information for students about aspects of recreation, ranging from camping and hiking to fishing and hunting. Students in the village were involved in numerous clubs and activities across campus. These included leadership roles in the Green Club, activities in CACTUS (student service), and the Giving Tree Project
- Undergraduates participating in the River village express high levels of satisfaction and engagement in learning about and acting on environmental concerns.

Presentations

American Association of Colleges and Universities Conference on Learning Communities (Providence, RI, March 2000). The River Village was included in a Poster presentation on Living Learning Communities at Tulane University.

Mississippi River Basin Alliance (Memphis, TN Sept. 2000)

The River Village co-hosted the Mississippi River Basin Alliance Conference at Tulane University in the Fall of 2001. The River Village made a presentation at the outset of the conference and hosted an open-house reception on the Saturday evening of the conference.

Dr. Kidder took several admissions trips, making presentations on the River Village to prospective students, and Drs. Wall, Kidder, and Roberts made presentations to prospective students during on-campus visits.

Dr. Koritz constituted and chaired the River Village Planning Committee beginning in January 2000. She assisted the lead instructors of the Mississippi River Basin Colloquium in conceptualizing and implementing a residence-hall based program centered around their course and in securing support and additional funding from the Office of the Senior Vice-President for Academic Affairs. Beginning in April 2000, Dr. Koritz moved into a consulting role, and the faculty directly involved in teaching the Mississippi River Basin Colloquium, Dr. Timmons Roberts, Dr. T.R. Kidder and Dr. Scott Wall, took over concrete planning and implementation tasks.

Intellectual Development

None.

Useable Technologies

None.

Environmental Studies

Principal Investigators: Christine Murphey, MSW
Program Coordinator
Las Cell and Molecular Biology
Tulane University

Co-Investigators: Timmons Roberts, Ph.D., Professor
Sociology Department
Tulane University

Joan Bennett, Ph.D., Professor
CMB
Tulane University

Reporting Period: September 1999 - February 2000

Primary Objectives of Educational Activities

Enhance environmental education at Tulane University through the establishment of Tulane as a national center of excellence in the environmental arena. Our efforts and accomplishments have proceeded in four areas: (1) continuing development of environmental studies faculty, (2) development of a major interdisciplinary course on the Mississippi River, (3) continuation of project for enhancing first year education for Environmental Studies students, and (4) involvement in a variety of additional Environmental Educational initiatives.

Progress Made to Achieve these Objectives

Several breakthroughs occurred within the Environmental Studies Program during this 18-month period. First, the academic curriculum expanded from one track to two: Environmental Science and Environmental Policy. Second, the Mississippi River Colloquium, after being taught for three years, became the framework of a new student living-learning community (the River Dorm). The Colloquium originally grew out of an Environmental Studies Faculty Seminar, and was sustained through the ENST budget prior to Spring, 2001.

Major Accomplishments

- Twenty-five new students attended the 1999 fall orientation. Sophomore Shannon Tanner showed slides from her summer work assisting with research on the swallow-tailed kite in the Atchafalaya Swamp. 160 new students signed an Environmental Action interest list at the September Activities Expo. 2000 Fall orientation was equally successful, which was followed by the Green Club's Environmental Action Week.
- Environmental Studies continues to participate in the Fall (1999 and 2000) Newcomb College Orientation and the Tulane College 'What's My Major' in Spring 2000.
- The number of Environmental Studies coordinate majors in fall, 1999 was 45.
- Christine Murphey visited Alliance for Affordable Energy in September, 1999 to discuss the possibility of student internships.
- Timmons Roberts (Sociology) replaced Michael Zimmerman as the new Environmental Studies co-Director in fall, 1999. Co-Director Joan Bennett resigned in the fall, 2000.

Tom Sherry (EEBiology), Jay Gullede (EEBiology), Sara Singleton (Political Science) and Liz Davey (Office of Environmental Affairs) joined the Environmental Education Committee.

- Five videos were purchased for Professor Roberts' Environmental Sociology class.
- Christine Murphey sent a letter to Environmental Studies Alumni (approximately 50 Tulane graduates) in fall, 1999, soliciting interest in mentorship of undergraduates and in receiving the Environmental Forum student newsletter.
- On October 22, 1999, David Barrett of BP in Belle Chasse presented two sessions to the Tulane community on obtaining ISO-14001 certification.
- In the Fall of 1999 Christine Murphey attended the LEAN conference in Baton Rouge and rode on the toxic tour led by Malik Wiley.
- The Mississippi River Colloquium was taught, for the second time, in the Spring of 2000, by Scott Wall (Architecture), Hank Bart (EEBiology) and TRKidder (Anthropology) to a full class of 25 students. Class field trips in the Mississippi River Colloquium included: Poverty Point and Belle Chase, Louisiana for water sampling of the river, Pilot Town and the River Toxic Tour (led by Malik Wiley). LUMCON, in Cocodrie, Louisiana, was the site of an overnight class trip, which included two boat excursions. The class ended in April with a public poster session. The Colloquium webpage has been updated with Spring, 2000, course additions (<http://www.museum.tulane.edu/mrbc/>).
- In Spring of 2000 a subscription to an on-line job listing service was purchased from the Student Conservation Association, and renewed in Fall, 2000.
- In Spring, 2000 graduating senior Environmental Studies Coordinate Major Hannah Carmalt won the first Stuart S. Bamforth Award for Academic Excellence.
- Environmental Studies Director Timmons Roberts, along with several other faculty members, met throughout the Summer, Fall (2000) and Spring (2001) to plan the educational component of the Mississippi River Dorm.
- In Fall of 2000 the Environmental Studies submitted its proposed Environmental Policy Track addition to the Environmental Studies Coordinate Major. The Curriculum Committee and the entire faculty approved the new track in December, 2000. Publicity advertising the new track included class visits and posters placed throughout campus.

Publications

Environmental Forum Newsletters were produced in the Fall, 1999, Spring, 2000 and Summer 2000.

Enviro-Counterculture Catalogs (produced by the Green Club) were available for distribution December, 1999 during registration for spring classes. A version of the catalog is posted on the web (<http://www.tulane.edu/~greencb/catalog>).

Links to student research, internship and job opportunities are listed on the Environmental Studies home page (http://www.tulane.edu/~env_stud) and e-mailed regularly as announcements to the Environmental Studies Majors.

Presentations

None.

Intellectual Development

1. **Student(s) Name:** Dan Au, Betsy Franke, Melissa Vernon, Christine Tanner, Shannon Tanner, Seth Willey, Rebecca Daniels, Dan Fruchter, Breck Baird, Mike Everett and Katie Kemp.
2. **Funding Period:** September 1999 to February 2001
3. **Duties and Responsibilities:** Melissa Vernon/Dan Fruchter newsletter and catalog editors; Betsy Franke/Rebecca Daniels office managers; Katie Kemp, Mike Everett, Christine and Shannon Tanner office workers, Dan Au accounting; Seth Willey/Breck Baird website and listserv administrators.

Useable Technologies

None.

Undergraduate Projects in Environmental Research and Design

Principal Investigator: Elizabeth Davey, Ph.D.
Program Manager
Center for Bioenvironmental Research
at Tulane and Xavier Universities

Reporting Period: September 1999 - April 2000

Primary Objectives of Research Activities

To develop an environmental education program that develops environmental problem-solving skills through research and design projects that reduce the university's environmental impacts and improve the local environment.

Progress Made to Achieve these Objectives

An application procedure for grants for student environmental research and design projects was developed and advertised, and a selection committee was formed. Grants were awarded for projects including: recycling system design for local schools, development of recycling education curriculum, a guide to campus trees, and testing local surface waters for pharmaceutical contaminants. Students were mentored and supervised by campus Environmental Coordinator. Results were presented to the campus community in April 2000 and April 2001, and reviewed and edited reports were posted on the internet at <http://www.tulane.edu/~caffairs/sturesearch.html>

Major Accomplishments

- Involved 18 students in intensive, active development of solutions to campus and local environmental problems. The program gave them direct experience with needs and opportunities in environmental fields, and practice in research and problem-solving skills.
- Provided environmental research and expertise to the New Orleans community. For example, the two recycling projects—development of a recycling curriculum and a method for designing school recycling systems—provide local school administrators and teachers with a complete program for introducing recycling into their schools. Copies are requested frequently, and the students are now working closely with the New Orleans Department of Sanitation to develop a district wide recycling teacher-training programs.
- Summaries and reports were edited and posted on the internet at <http://www.tulane.edu/~caffairs/asa.html>
- Increased the larger community's understanding of environmental issues and solutions:
- The April 2000 final presentations and poster session drew audience of 70 (mostly students) and the April 2001 drew over 50.
- Recycling education and design programs involved over 100 local elementary and high school students.
- Tulane community will use the tree guide as a reference for the next several years.

- Over 20 Tulane faculty and staff from across the university involved in the program as advisors, including some non-traditional advisors, such as the grounds superintendent, the campus architect, and director of the Herbarium.

Publications, Manuscripts, Abstracts

None.

Presentations

None.

Intellectual Development

1. **Student Name(s):** Eman Williams
 2. **Funding Period:** Nov 1999 to May 2000
 3. **Duties and Responsibilities:** Developed and taught an eight-week, hands-on recycling curriculum for elementary school students. The curriculum is available at <http://www.tulane.edu/~eaffairs/williamslessons.pdf>
-
1. **Student Name(s):** Rachel Nelson
 2. **Funding Period:** Nov 1999 to Jan 2001
 3. **Duties and Responsibilities:** Created an 11" x 17" guide to trees in selected Tulane campus landscapes, emphasizing differences between native and exotic tree species.
-
1. **Student Name(s):** Nikki Thanos
 2. **Funding Period:** Jan 2000 to May 2000
 3. **Duties and Responsibilities:** Summarizing and printing her undergraduate thesis on sustainable development in Louisiana and Costa Rica for a non-academic audience.
-
1. **Student Name(s):** Kristen Smeby
 2. **Funding Period:** Jan 2000 to May 2000
 3. **Duties and Responsibilities:** Sampling and analysis of pharmaceutical contaminants in Lake Pontchartrain and the Mississippi River.
-
1. **Student Name(s):** Gretchen Thompson, Shelly Moczygemba, Mark Huthmaker, Ayse Ercuemen, Matthew Pang, Erin Lerhner, Yojna Singh, Jason Mellad.
 2. **Funding Period:** Jan 2000 to April 2001.
 3. **Duties and Responsibilities:** Designing sustainable recycling systems for local elementary schools. Worked with St. Stephens in 2000 and McMain High School in 2000-01.
-
1. **Student Name(s):** Marcela Casas
 2. **Funding Period:** Jan 2001 to April 2001.
 3. **Duties and Responsibilities:** Monitoring interior airflows, light and occupant comfort in selected locations at Tulane University.

1. **Student Names:** Alexandra Cervenka
2. **Funding Period:** Jan 2001 to April 2001
3. **Duties and Responsibilities:** Assisting with the creation of the New Orleans Bike Map, including learning ArcView software, tracing bicycle routes onto a base map, and ground truthing suggested routes.

1. **Student Names:** Jennifer Karam, Shelley Kahler, Alana Paul, and Maureen Devery
2. **Funding Period:** Nov 2000- Feb 2001.
3. **Duties and Responsibilities:** Conducting a greenhouse gas emissions inventory of the Tulane University Uptown campus and researching policies and programs for reducing emissions.

Useable Environmental Technologies

None.

Education and Communication Core

SPRITE: The Summer Pipeline Research Initiative: the Tulane Experience A Mentored Introduction to Programs of Study for Graduate Research

Principal Investigator: Valerie Petit Wilson, Ph.D.
Clinical Associate Professor
Environmental Health Sciences
Deputy Director, Center for Bioenvironmental Research
at Tulane and Xavier Universities

Co – Investigators: Charles E. Allen, III, MSPH
Education and Outreach Coordinator
Center for Bioenvironmental Research

Dana M. Greene-McDowelle, Ph.D.
Assistant Professor, Biology
Xavier University of Louisiana

Reporting Period: May 2000 - April 2001

Primary Objectives of Research Activities

The Summer Pipeline Research Initiative: the Tulane Experience (SPRITE) is an educational initiative that the Center for Bioenvironmental Research (CBR) coordinates between Tulane University's (TU) Molecular and Cellular Biology (MCB) Graduate Program and Xavier University's (XU) Office of Sponsored Programs. The goal of SPRITE is to increase the number of minorities at the graduate level in the bioenvironmental and biomedical sciences by:

- Providing Xavier undergraduate students with a quality bench research experience in an MCB laboratory under the guidance of an established researcher; and
- Exposing these students to graduate life and the MCB program of Tulane University.

The program focuses on two unique resources: Tulane's successful biomedical graduate program and Xavier University's outstanding pool of science majors. The intent of the program is to provide a mentored introduction to Tulane's excellent programs of graduate study with a successful research experience partially supported by ONR.

In addition to the research, students also participate in weekly seminars and roundtable discussions led by the SPRITE program staff. These seminars cover areas such as research at the frontiers of science, financial aid and career opportunities. In addition, students are provided with exposure to scientific ethics using the National Academy of Sciences Text "*On Being A Scientist*" in addition to a lecture/discussion on biomedical ethics. Other sessions include forums on the presentation of a research seminar and training on graphics and presentation skills.

The culminating event for the summer activities is a research symposium in which student interns present their work to the assembled faculty, students, laboratory colleagues and staff. Subsequently, in the following fall, students have access to SPRITE staff for assistance in applying to graduate and professional schools for the appropriate academic year.

Progress Made to Achieve These Objectives

- The SPRITE program originated in June 1999 with an initial six students. At the end of the summer experience, the informal evaluations of the programs from the mentors, students and administrators suggested that the format of the trial program was beneficial. This initial trial was highlighted by the acceptance of one of the students into the Tulane MCB graduate program.
- For summer 2000, program coordinators developed application packets and disseminated information to eligible sophomores, juniors, and seniors at Xavier University. Presentations were made to faculty and students at Tulane and Xavier Universities to recruit both mentors and research interns.
- In summer 2000, six students were competitively selected from a pool of 24 applicants for the 10-week summer program.
- The summer 2000 program involved faculty research mentors from the following disciplines: Chemistry, Microbiology/Immunology, Ophthalmology, Pharmacology, Psychology, and Tropical Medicine
- Three SPRITE interns from the 2000 summer program attended the Tuskegee University Undergraduate Science and Engineering Conference that was held October 20-22, 2000.
 - **Randi Smith** presented her project entitled *Detection of Lead by Immunoassay*, conducted under the guidance of Diane Blake, PhD, Associate Professor, Department of Ophthalmology, Tulane University Health Sciences Center (TUHSC). Randi Smith won first place in her division of Chemistry and Chemical Engineering.
 - **Cecily Jones** presented her project entitled *Ecological Remediation: Resurrection of an Extinct Frog Species from Puerto Rico*, conducted under the guidance of Scott Michael, PhD, Assistant Professor, Department of Tropical Medicine, TUHSC. Cecily placed second in her division of Agricultural, Biological and Environmental Sciences.
 - **Tametra Johnson** also attended and presented her work at the conference. Her project entitled "*Serotyping of Pseudomonas aeruginosa*" was conducted under the guidance of Michael Schurr, PhD, Department of Microbiology and Immunology, TUHSC.
- Activities by the SPRITE coordinators in fall 2000 included assistance with student applications to graduate school and other professional schools.
- In spring 2001, two of the summer 2000 students were accepted to Tulane Medical School, another one into the Tulane Molecular and Cellular Biology Program, and the fourth into Tulane School of Public Health and Tropical Medicine. The remaining two students were entering their final year of undergraduate school (one subsequently was accepted into a Ph.D. program at Emory University in Spring 2002).
- Selection for SPRITE interns for the summer 2001 program was equally competitive; more than 26 applications were received for the 6 positions. Funding for this set of interns was shared between ONR (2001 funding) and Xavier University's Model Institutions of Excellence (NSF project grant).
- The success of SPRITE provides a national model for pipeline partnerships between research institutions and historically black colleges/universities.

Publications

None

Presentations

- By Students - Tuskegee University, October 20-22, 2000, The Tuskegee University Undergraduate Science and Engineering Conference (see above for description).
- By Students - Louisiana Alliance for Minority Participation Undergraduate Research Conference, February 7, 2001
- By ONR Faculty – Valerie P Wilson, Ph. D. “*SPRITE Program: Research Mentoring as a Key to Increasing Minority Enrollment in SEM Fields*” at Roadmap to Opportunities Conference, April 25-26, 2001.

Intellectual Development

1. **Student Name:** Katrina Dunson
2. **Funding Period:** May through August 2000
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled “*Co-localization of Fos B and mu-receptors in the medial peroptic area of postpartum rats*”
4. **Research Advisor:** Dr Cynthia Gulledge, Center for Bioenvironmental Research

1. **Student Name:** Tametra Johnson
2. **Funding Period:** May through August 2000
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled “*Serotyping of Pseudomonas aeruginosa*”
4. **Research Advisor:** Dr. Michael Schurr, Microbiology and Immunology, TUHSC

1. **Student Name:** Cecily Jones
2. **Funding Period:** May through August 2000
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled “*Ecological Remediation: resurrection of an extinct frog species from Puerto Rico*”
4. **Research Advisor:** Dr. Scott Michael, Tropical Medicine, TUHSC

1. **Student Name:** Randi Smith
2. **Funding Period:** May through August 2000
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled “*Detection of lead by immunoassay*”
4. **Research Advisor:** Dr Diane Blake, Ophthalmology, TUHSC

1. **Student Name:** Danielle Williams
2. **Funding Period:** May through August 2000
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled “*The effects of chelerythrine on social transmission*”
4. **Research Advisor:** Dr. Paul Columbo, Psychology, Tulane University

1. **Student Name:** Kendra Jupiter
2. **Funding Period:** May 2000 – August 2000*
3. **Duties and Responsibilities:** Student was responsible for conducting research on the project entitled “*Helicity of Lyme Disease peptide Antigens*”

4. Research Advisor: Dr. Pernilla Wittung-Stafshede, Chemistry, Tulane University

*Kendra Jupiter was subsequently accepted into and enrolled in the Tulane University Molecular and Cellular Biology Graduate Program in Fall 2000.

Useable Technologies

None

Foundations of a five-year program between Xavier Physics/Engineering and Tulane leading to a master degree

Principal Investigator: Elia Eschenazi, Ph.D.
Associate Dean, Physics and Engineering
Xavier University

Co-Investigator: Stathis Michaelides, Ph.D.
Associate Dean, Engineering
Tulane University

Reporting Period: May 1999 – April 2001

Primary Objectives of Research Project

This project proposes the establishment of the foundations of a five-year program leading to a master degree in engineering from Tulane University and BS in Physics / or Chemistry at Xavier University. This program will allow the students in the Departments of Physics/Engineering at Xavier to complete the requirements for both the bachelor's degree and the master's degree in the School of Engineering at Tulane within five years from their matriculation. Summer research work will be required for the timely completion of the master's thesis.

The purpose is to continue consolidating and enhancing the pipeline producing more African American masters and PhDs. In the development of the program students will be involved for two summers of their 3rd and 4th year in research projects which will lead to their master thesis. This not only will create a substantial basis for the successful development of the program but it will also foster the collaboration of the Universities in research areas relevant to the Office of Naval Research.

Progress Made to Achieve these Objectives

- Three students were initially accepted in the program beginning their 4th year at Tulane in the fall '00. Two students in mechanical engineering, Lorenzo Craig and Melodie Wyche and one student in chemical engineering, Monique Gibbs.
- In the summer '00 the three thesis projects were assigned. The two mechanical engineering students were mentored by Dr. Eschenazi and Dr. Michaelides and chemical engineering students by Dr. Eschenazi and Dr. Papadopoulos
- The students enrolled in graduate courses in the Spring '01 and they spent the second research summer on their thesis project in the summer '01 under the supervision of the same faculty members. Good progress was made in the theses projects.
- These students are expected to graduate in the summer '02 as planned in the program.
- There are plans to add two-three more students to the program by the Fall '01.

- Progress has been made towards the “institutionalization” of the program. A committee created by the Deans and including the two co-pis of the project is in the process of developing an articulation agreement between the two Universities.
- The team effort of Xavier/Tulane faculty in mentoring the students and the strong collaboration in the various research projects is consolidating the training/research environment and the partnership, which are fundamental ingredients for the success of the program.

Publications

None.

Presentations

None.

Intellectual Development

1. **Student(s) Names:** Lorenzo Craig, Melodie Wyche, Monique Gibbs.
2. **Funding Period:** May 1999 to April 2001
3. **Duties and Responsibilities:** Students were responsible for the following theses and projects assigned:

Lorenzo Craig: “ *Influence of suspended particles on the hydrodynamic force acting on a stationary particle*”

Monique Gibbs: “ *Adsorption of Humic Acid in the Presence of Al³⁺ and its Effects on Colloid-Facilitated Transport* ”

Melodie Wyche: “ *Influence of Basset force on the dynamics of particles in unsteady, periodically driven flows*”

Useable Technologies

None.

Cooperative Army Corps of Engineers

Principal Investigator: Colin M. MacLachlan, Ph.D.
Professor
History Department
Tulane University

Co-Investigator: Edwin Lyons
Adjunct Professor
History Department
Tulane University

Reporting Period: August 1999 – May 2001

Primary Objectives of Research Project

The objective of this project is to set up cooperative projects with the New Orleans District – Corps of Engineers and Tulane University including information transfer, internships and graduate training. General support for planning of the Mississippi River Museum.

Major Accomplishments

Graduate education

- Completed development of joint Ph.D. program in history and historic preservation.

Internships

- Facilitated Career Services internship agreement with Corps of Engineers.
- Located internship opportunities at organizations such as the National Park Service in New Orleans and Atlanta, New Orleans Pharmacy Museum, Notarial Archives, Corps of Engineers, Minerals Management Service, Evergreen Plantation, Historic New Orleans Collection. These opportunities are for students in English, Biology, Environmental Studies, History, Historic Preservation, Theatre, Women's Studies.
- Wrote draft internship section for undergraduate EEOB handbook.

Mississippi River

GENERAL

- Supported development of the Randal L. Gibson conference on Mississippi River.

GIS

- Developed Geographic Information System project with CBR. CBR funded students assisted in inventory of spatial data at Corps of Engineers. CBR scanned maps to include in joint Tulane-Corps of Engineers GIS. Met with faculty in a number of departments to discuss GIS issues: EEOB, Geology, History, Civil Engineering, CBR.

Mississippi River Museum

- Prepared proposal for grant from Corps of Engineers Planning Assistance to States program.
- Prepared Museum conference proposal.

- Supported efforts of Mississippi River Museum planning group in numerous ways. For example, identified consultants to plan a three year plan for Museum development.

Tulane-COE relationship

General

- Established relationships with many Tulane faculty members. Addressed a number of faculty meetings including LAS Chairs and Directors, EEOB faculty, and Anthropology Department.

Payson Center

- Worked to develop a relationship between the Disaster Relief and Humanitarian Relief program of the Payson Center and the Corps of Engineers

Reduced tuition graduate program

- Established a reduced tuition rate for New Orleans District employees to take graduate courses at Tulane.

Faculty Research at Corps of Engineers

- Discussed faculty research opportunities with faculty in EEOB and geology.

Tulane-Other Organizations relationships

National Park Service

- Developed relationship with Southeast Region of National Park Service in Atlanta. Included grant for interns in historic preservation program for National Landmark field visits. A Preservation student worked as an intern on a National Landmark nomination for the Louisiana State Hospital. A National Park Service participated in the spring Historic Preservation meeting organized by Gene Cizek.

New Orleans Public Schools

- Organized meeting with the Students in the Center program of the New Orleans Public Schools. Attended by faculty from psychology, English. Staff from CBR

Meetings attended

- Mississippi River Parkway Commission meeting
- Mississippi River Road Commission
- Lower Mississippi River American Heritage River working group

Information Transfer

- Identified information at Corps of Engineers for numerous faculty members and students.
- Served as guest lecturer for Tulane courses.
- Supported Mississippi River Basin Colloquium

Publications: None

Presentations: None

Intellectual Development: None

Useable Technology: None

**Environmental Hormone Symposium Series
(e.hormone), 1999 – 2001**

Principal Investigator: John A. McLachlan, PhD
Weatherhead Distinguished Professor and Director
Center for Bioenvironmental Research
At Tulane/Xavier Universities

Reporting Period: August 1999 – April 2001

Primary Objectives of Research Project

One of the central themes of the CBR's Integrated Bioenvironmental Hazards Research Program is understanding how bioenvironmental contaminants can impact the health of humans and wildlife and their progeny through disruption of the endocrine system. Understanding the many issues surrounding environmental endocrine disruption, or environmental signaling (eg. contaminants and pollutants) and its effects on human and ecosystem health requires a synthesis of disciplines ranging from molecular biology to systemic population biology. This becomes a daunting task since the scientific terminology and methodology, the meetings attended, and literature read by researchers does not in many cases overlap. The CBR responded to the need for a scientific forum for information exchange and collegial interaction for scientists involved in environmental endocrine research by hosting the first international Environmental Hormone Symposium (e.hormone) in October 1999.

Now in its fourth year, the e.hormone annual symposium provides the only venue for discussions of basic and applied research into this critical topic with the breadth and depth they require. ONR-funded researchers who have presented at this symposium include Drs. Diane Blake, Glen Boyd, Doug Meffert, Valerie Wilson, and John McLachlan. Dr. Tom Wiese, another ONR researcher, coordinates and chairs each poster session. Formal and informal networking opportunities are built into the schedule of e.hormone symposium activities. Information exchange is enhanced via discussion periods after each formal session, and an extensive poster session. Evening social functions provide a relaxed, informal context different from the rigid daytime program schedule.

The goal of this annual symposium is to bring together innovative thinkers, cutting edge researchers, and key decision makers to critically evaluate current research on endocrine disruption and contribute to the future of this new field. e.hormone has brought about a synthesis of ideas and disciplines to address the important issues raised in the area of scientific inquiry related to environmental signaling.

The e.hormone symposium is an annual forum for exchange of new, often unpublished information, and multidisciplinary, multinational scientific interaction. Ecologists, chemists, endocrinologists, toxicologists, zoologists, engineers, philosophers, undergraduate science faculty, high school teachers, policy makers, and media from the United States, Japan, Europe, and Latin America meet yearly to analyze the latest findings on environmental hormones and signaling that are the basis of endocrine disruption. The meeting is the cutting edge in research and policy.

Educational Objectives: After attending this continuing education activity, a participant should be able to:

- ❑ Interpret cutting edge research and techniques related to environmental hormones and apply current knowledge to future research or decision-making.
- ❑ Identify research interests and colleagues in the field of environmental hormones that will foster future collaboration or information exchange
- ❑ Understand philosophical approaches, concepts, frameworks, and policy implications related to endocrine disrupting chemicals.

Progress Made to Achieve these Objectives

e.hormone has become the focal point for all those who are interested in the field of environmental signaling. Sessions are held at the CBR conference facility in the Health and Environmental Research Building in downtown New Orleans. The symposium format includes scientific presentations grouped around conceptual themes. The organizing committee, cognizant of the latest in literature and findings, carefully selects presenters who are conducting cutting edge research across a variety of disciplines and represent diversity in race/ethnicity, gender, geographic, and senior/junior research status. Preminent experts in the field introduce sessions and provide historical perspective on their topic and highlight recent findings.

Examples of presentations on ONR-related research topics and themes follow. During the e.hormone 1999 symposium, Dr. Ann Cheek (Southeastern Louisiana University), a CBR DoD 1996 investigator, presented "Thyroid hormone activity of environmental chemicals and biological consequences for aquatic vertebrates," and Dr. Koji Arizono (Prefectural University of Kumamoto) presented "The screening of environmental estrogens using the worm, *C. elegans*" in the **Hormonal Signals and Networks in the Environment** session. During the **Hormones as Chemicals, Chemicals as Hormones** session, Dr. John Katzenellenbogen (University of Illinois) presented "Estrogenic chemicals: twenty years of structural biology," and Dr. William Toscano, a CBR DoD 1996 investigator, presented "Dioxin and estrogen receptor systems: an unfriendly repartee?"

During the e.hormone 2000 symposium, Dr. Maria Fossi (Universities of Messina and Sienna, Italy) presented "Biomarkers as diagnostic and prognostic tools for assessing effects of endocrine disrupters among top predators in the Mediterranean ecosystem," and Dr. Glen Boyd, a CBR ONR researcher, presented "Occurrence of pharmaceutical contaminants and screening of treatment alternatives for Southeastern Louisiana" in the **Hormones and Ecosystem Change** session. During the **Environmental Hormones & Biological Change** session, Dr. Gerald LeBlanc (North Carolina State University) presented "Invertebrate endocrine responses to environmental signaling," and Dr. Ian Callard (Boston University) presented "*C. elegans* as a model for environmental change."

Major Accomplishments

Throughout its three-year history, the e.hormone symposium has resulted in the creation of an extensive global network. Major accomplishments include:

- ❑ Increased participation – attendance rose from 89 in 1999 to 136 in 2000
- ❑ Symposium continuity - many returning 1999 attendees as well as new faces in 2000

- ❑ Comprehensive poster session for junior investigators - 26 posters featured in 1999 and 39 posters in 2000
- ❑ A global approach to science – 4 of the 24 speakers in 1999 were international, and 13 international attendees; 7 of the 26 speakers in 2000 were international, and 19 international registrants
- ❑ Growing research relationships with Japanese colleagues – 9 attended in 1999, 14 in 2000
- ❑ A "spin-off" e.hormone website as a hub of scientific and media information connecting research colleagues throughout the year
- ❑ Creation of a mentoring/networking forum for junior investigators – 43 students and fellows registered in 1999; 54 in 2000

Publications

Each of the past three symposia has been reported on the web, and its scholarship recognized in publications such as *Science News*. The proceedings from e.hormone 2000 were published as a volume in *the Annals of the New York Academy of Sciences*

McLachlan JA, Guillette LJ, Iguchi T, Toscano WA, eds. "Environmental hormones: The scientific basis of endocrine disruption." *Annals of the New York Academy of Sciences*. Vol. 948, pp. 1-143.

Presentations

e.hormone 1999 sessions:

- I Hormonal Signals and Networks in the Environment
- II Hormones as Chemicals, Chemicals as Hormones
- III Mechanisms of Hormone Responses
- IV Developmental Toxicology of Hormonally Active Chemicals
- V Focus on Environmental Hormones: Where Do We Go From Here?

e.hormone 2000 sessions:

- I Hormonally Associated Disorders in Men & Women
- II Hormones & Ecosystem Change
- III Molecular Mechanisms of Sex Differentiation
- IV Environmental Hormones & Biological Change
- V Environmental Hormones: a Philosophical View
- VI Newly Emerging Targets & Interactions for Environmental Hormones

e.hormone 2001 sessions:

- I Environmental Factors & Human Sexual Development
- II Sex Reversal – Fish
- III Hormonally Active Agents in the Environment
- IV Mechanisms of Environmental Hormone Action
- V Hormones in Male Reproductive Tract Development
- VI Environmental Estrogen Factors & Breast Disease
- VII Federal Programs in Endocrine Disruption - Priority Needs & Future Directions

Intellectual Development

N/A

Useable Environmental Technologies

While no technologies have resulted directly from the e.hormone workshop series, the CBR deems that interdisciplinary workshops like this one are critical for the creation of scientific collaborative approaches that foster biosensor development. Such biosensors harness the power of "environmental signaling," and lead us to further exploration and development of numerous near real-time monitoring technologies for the ONR, in particular, and the DOD, in general.

ENVIRONMENTAL SIGNALS AND SENSORS

Quantitative Structure Activity Relationships from Molecular Dynamics: A Computational Method of Identifying Environmental Estrogens

Principal Investigator: Thomas C. Bishop, Ph.D.
Assistant Professor
Environmental Health Sciences
Tulane University Health Sciences Center

Co-Investigator(s): Thomas E. Wiese, Ph.D.
Assistant Professor
Environmental Health Sciences
Tulane University Health Sciences Center

Reporting Period: July 1999 – April 2001

Primary Objectives of Research Activities

The primary objective was to combine QSAR and MD to create a new method of analysis of ligand-receptor interactions. The specific aims were:

1. Combine QSAR and MD to create a new method of analysis of ligand-receptor interactions, QSAR-MD, that will be used to study the interaction of xenoestrogens with the human estrogen receptor α (hER- α).
2. Apply QSAR-MD to study the interaction of xenoestrogens with a wildlife species of ER, rainbow trout ER (rtER).
3. Compare the QSAR-MDs of hER- α and rtER to determine if different ligand-receptor interactions have evolved for these two species of receptor.

Progress Made to Achieve these Objectives

1. Long time scale molecular dynamics simulations of DES and Estradiol bound to the estrogen receptor ligand-binding domain.
2. Molecular dynamics annealing of estradiol bound to the estrogen receptor ligand-binding domain.
3. Parameterization of the test-10 compounds.
4. Molecular models of 12 mutant structures of the estrogen receptor ligand-binding domain.

Major Accomplishments

- **Long time scale molecular dynamics simulations of DES and Estradiol and Annealing Simulations.** Twelve 1.6ns simulations using six different conformations of the ER-LBD with DES bound and with Estradiol bound have been conducted. Analysis of the results is focused on the characterizing the dynamics of the ligand within the binding cavity and is ongoing. These detailed simulations will provide the necessary background and control information for simulating the 10 test compounds. The

annealing simulations were conducted as part of the experimental control to determine the stability of the ligand-receptor complex during

- **Parameterization of the test-10 compounds by ab initio quantum mechanics.** *Ab initio* (HF/6-31G*) and semi empirical (AM1) optimization and electrostatic potentials calculations have been for all of the ten test compounds to be simulated. The calculations used for assigning partial charges to the ten compounds according to the RESP methodology used for the Werner et.al. force field in AMBER. Each of the ten different molecules to be docked to the receptor has been modeled using three different levels of molecular modeling: semi-empirical quantum mechanics optimization (AM1, PM3), *ab initio* quantum mechanics optimization (HF/6-31G*), and molecular mechanics (Sybyl and Amber Force Fields). And the results compared to determine the validity of our choice of molecular mechanics parameters.
- **Molecular models of 12 mutant structures of the estrogen receptor ligand-binding domain.** Twelve different mutant structures of the estrogen receptor ligand-binding domain have been modeled. These structures were chosen because they have been experimentally well characterized for their effects on the relative binding of estradiol to the receptor. Each mutant structure has a relative binding affinity for estradiol that differs by more than a factor of 10 from wild type. These structures will be used to further assess the properties of the ligand receptor interactions.

Publications, Manuscripts, Abstracts

Marhefka, Craig A., Moore II, Bob M., **Bishop, Thomas C.**, Kirkovsky, Leonid, Mukherjee, Arnab, Dalton, James T., and Miller, Duane D. Homology Modeling Using Multiple Molecular Dynamics Simulations and Docking Studies of the Human Androgen Receptor Ligand Binding Domain Bound to Testosterone and Nonsteroidal Ligands. *J. Med. Chem.* 44, 1729-1740, 2001.

Bishop, Thomas C., Williams, Kirk Y., Hall, Andrew. Dynamics of Estradiol and DES in solution and bound to the estrogen receptor. (in preparation)

Sadaka, Marc Masters Degree Student, Dept. Environmental Health Science, Tulane University. The Advance of Molecular Techniques: Computer Simulation of Small Molecules. Submitted in partial fulfillment of the requirements for Master of Science Degree

Basavapathruni, Radha Masters Degree Student, Dept. Environmental Health Science, Tulane University. Molecular Docking and Molecular Dynamics Simulations of Endocrine Disrupting Chemicals. In preparation for submission as partial fulfillment of the requirements for a Master of Public Health

Presentations

Seminar: "Molecular Models of Xenoestrogen Activity" University of New Orleans, New Orleans, LA. Department of Chemistry October, 1999.

Computer Demonstration: "Interactive Molecular Dynamics of the Estrogen Receptor", Environmental Hormones: Past, Present, Future, October 18-20, 1999. Center for Bioenvironmental Research, New Orleans, LA. Thomas C. Bishop

Computer Demonstration: "Opening the Door for Estrogens", Environmental Hormones Conference. October 15-18, 2000. Center for Bioenvironmental Research, New Orleans, LA. Thomas C. Bishop and Andrew Hall.

Seminar: "Filling the Estrogen Receptor Binding Cavity", Environmental Signaling and the CNS, a satellite symposium of the Society of Neuroscience 30th Annual Meeting. November 4, 2000. Center for Bioenvironmental Research, New Orleans, LA.

Seminar: "Three Dimensional Model of the Human Androgen Receptor Ligand Binding Domain Bound to Testosterone and Non-Steroidal Ligands". Mississippi-Alabama-Louisiana-Tennessee-Oklahoma (MALTO) meeting of the Schools of Pharmacy, May 21-23, 2000. New Orleans, LA. Craig Marhefka, Thomas C. Bishop, Bob Moore II, James Dalton, and Duane Miller.

Intellectual Development

1. **Student Name(s):** Shakia Molier-Davis and Nicole Hunt
undergraduate Xavier University and graduate student Tulane MCB program.
 2. **Funding Period:**
 3. **Duties and Responsibilities:** Analyze and mutants of hER-a LBD use molecular modeling techniques. These were responsible for searching the literature for structural information regarding mutants of the human estrogen receptor. They then used molecular modeling tools to introduce these mutations into the known x-ray crystallographic structures of these receptors and analyze the results.
-
1. **Student Name(s):** Lena Gamble, Junaia Carter, and Kendria Hall: undergraduates Xavier University
 2. **Funding Period:** Fall 1999
 3. **Duties and Responsibilities:** Enter data and construct three-dimensional models of ten different known endocrine disrupting chemicals using SYBYL, a suite of modeling tools available from Tripose, Inc.
-
1. **Student Name(s):** Mark Sadaka, a graduate student Tulane University Environmental Health Sciences.
 2. **Funding Period:** Fall 1999-Spring 2000
 3. **Duties and Responsibilities:** Mark was responsible for analyzing the models created by Lena, Juanai, and Kendria. For this purpose he compared structures of the ten different molecules obtained by three different levels of molecular modeling: semi-empirical quantum mechanics optimization (AM1,PM3), ab initio quantum mechanics optimization (HF/6-31G*), and molecular mechanics (Sybyl and Amber Force Fields)

1. **Student Name(s):** Radha Basavapathruni, Graduate student Tulane University Environmental Health Science.
2. **Funding Period:** Spring 2000
3. **Duties and Responsibilities:** Radha was responsible for constructing models of 100 different known endocrine disrupting chemicals and conducting an extensive literature review of molecular docking and dynamics techniques as specifically applied to hormone receptor ligand binding domains.

Useable Technologies

None.

Mathematical Models of Signaling

Principal Investigator: Tom Bishop, Ph.D.
Assistant Professor
Environmental Health Sciences

Co-Investigator(s): Oleksandr Zhmudsky, MS
Research Scientist
Center for Bioenvironmental Research

Reporting Period: August 2000 - July 2001

Primary Objectives of Research Activities

Develop mathematical and computational models of signaling through DNA and chromatin.

Progress Made to Achieve these Objectives

1. Determination of analytic solutions to the linearized equations of motion governing the dynamics of an elastic rod (i.e. DNA and chromatin).
2. Numerical analysis of the equations of motion and comparison the analytic solutions obtained in 1).
3. Numerical analysis of the rate of propagation of mechanical disturbances through bent DNA to determine the relationship between bending of DNA and the rate of propagation of mechanical disturbances.
4. Analysis of a simple model for the folding of DNA into extended and condensed chromatin to determine how folding relates to dynamics.

Major Accomplishments

- **Linear Analysis:** Enabled identification of four different types of mechanical disturbances that can propagate through an elastic rod and determination of the speed of propagation of these types of disturbances based only on the elastic properties of the fiber. The four types correspond to an extension, bend, twist, or shear motion of the rod. Twist and extension motions are unique in that they can propagate along an elastic rod independently. The fact that twist can propagate along an elastic rod, such as DNA, without affecting any other mode has direct application to the analysis of the transcription and replication. Knowing the rate of propagation of twist allows us to analyze the process of twist relaxation and diffusion through DNA. This theory is scalable so it applies equally to macroscopic objects such as cables and beams, microscopic objects such as hair and cilia, as well as, molecular objects, such as actin filaments and DNA.
- **Numerical Simulations:** Have enabled the determination of the limits of the linear theory, in particular the linear theory is only applicable for the case of unbent DNA. Using numerical simulation techniques we demonstrated that when DNA is uniformly bent to the same degree that it is bent in the nucleosome, that the speed of propagation of twist and extension will be altered by as much as 5%.

- Simple Model of Folding: The higher order folding of DNA into extended and condensed chromatin is readily observed but the details of the folding cannot be unambiguously determined experimentally. We have determined elastic constants for a simple fold geometry of DNA and compared them to experimental measurements of the elastic properties of extended and condensed chromatin. The model suggests that the elastic properties of extended chromatin can be deduced by a simple helical folding of DNA while the properties of condensed chromatin cannot be directly related to the so-called solenoid model based on only the elastic properties of DNA.

Publications, Manuscripts, Abstracts

Bishop, Thomas C and Zhmudsky, Oleksandr O. Mechanical Model of Nucleosome and Chromatin Dynamics (submitted)

Bishop, Thomas C and Zhmudsky, Oleksandr O. Elastic Wave Propagation Along DNA. Los Alamos National Labs E-Print Archive. arXiv:physics/010171 v3 .

Bishop, Thomas C and Zhmudsky, Oleksandr O. Information Transmission along DNA. *Currents In Computational Molecular Biology 2001*. Pg 105-106. Les Publications CRM, Montreal. Eds. N. El-Mabrouk, T. Lengauer, and D. Sankoff.

Presentations

Poster: "Dynamics of DNA Depends on Conformation: An Implied Communications Network", Intelligent Systems in Molecular Biology, August 20-23, 2000. La Jolla, CA. Thomas C. Bishop, Y.M. Shi and J.E. Hearst.

Seminar: "DNA a Cellular Communications Network." Tulane University Department of Mathematics. Thomas C. Bishop

Poster: "Information Transmission Along DNA", RECOMB 2001: The 5th Annual International Conference On Computational Molecular Biology, April 22-25, 2001. Montreal, Canada. Thomas C. Bishop and Oleksandr Zhmudsky.

Poster: "Approximate Dynamics of DNA, the 11nm and 30nm Fibers", Albany 2001: The Twelfth Conversation, June 19-23, 2001. Albany, NY. Thomas C. Bishop and Oleksandr Zhmudsky.

Intellectual Development: None

Useable Technologies: None.

Antibody-Based Biosensors for Autonomous Underwater Vehicles

Principal Investigator: Diane A. Blake, Ph.D.
Associate Professor
Department of Ophthalmology
Tulane University Health Sciences Center

Co-Investigators: George C. Flowers, Ph.D.
Associate Professor and Chair
Department of Geology
Tulane University

Robert C. Blake II, Ph.D.
Professor and Interim Chair
Department of Basic Pharmaceutical Sciences
Xavier University of Louisiana

Reporting Period: August 1999 - July 2001

Summary of Progress:

Primary Objectives of Research Project

The goal of this project is to develop a biosensor that will permit the rapid, automated identification and quantification of EDTA in river water. A set of high-affinity, highly selective binding reagents (antibodies) will be used to develop an immunosensor for EDTA that can operate in an autonomous underwater vehicle (AUV). The specific aims for this project are to 1) isolate and characterize monoclonal antibodies that bind tightly and specifically to EDTA. 2) test antibody activity in the environmental sample matrix (Mississippi River water); and 3) direct the progress of Sapidyne Instruments to construct a prototype immunosensor that can be deployed in an AUV.

Major Accomplishments

- The first assay to be developed will be for EDTA. Three different monoclonal antibodies have been developed for EDTA. Two antibodies (1B11 and 2D42) show unusual cooperative binding behavior. A third antibody (4B33, which binds to non-cooperatively EDTA with nanomolar affinity) will be used for subsequent sensor development. In addition, 15 different, well-characterized monoclonal antibodies are available for these immunosensor studies.
- A new method for producing large quantities of monoclonal antibody in a tissue bioreactor has been developed. The genetic stability of several different hybridomas under culture in this device has been determined.

- Negotiations/purchase orders for the construction of the immunosensor were finalized with Sapidyne Instruments, Inc., Boise, ID. Three full-size mock-ups of the submersible device were delivered to Tulane University in January 2000.
- One of the mock-up sensors has been returned to Sapidyne for installation of optical components.
- Sapidyne has designed software and plumbing modifications that will allow Xavier and Tulane scientists to test fluidics design of the AUV-based sensor on a research grade KinExA 3000. These hardware and software modifications have been delivered and installed on a KinExA 3000 instrument at Xavier University.
- A prototype assay for EDTA, the first analyte to be developed for this instrument, has been established on the KinExA 3000 research instrument.
- Mississippi River water samples have been subjected to geochemical analysis in preparation for further assay development.
- An antibody specific for chelated complexes of Pb (II) has been expressed as a recombinant protein. Molecular modeling and site-directed mutagenesis have implicated lysine 58 in the heavy chain as important for antigen recognition.

Publications, Manuscripts and Abstracts:

J.B. Delehanty, M. Khosraviani, R.C. Blake II, H. Yu, and D.A. Blake (2001) Recognition of Pb (II)-chelate complexes by a monoclonal antibody and its F_{ab} fragment, *Bioconjugate Chem.*, submitted.

J.B. Delehanty, H. Yu, and D.A. Blake (2001) Lysine 58 in the heavy chain of a monoclonal antibody specific for chelated complexes of lead is important for antigen recognition. *J. Biol Chem.*, submitted.

A.M. Kriegel, H. Yu, I.A. Darwish, R. Srinivasan, and D.A. Blake (2002) Production of mouse and rat monoclonal antibodies and stability of hybridoma cultures in membrane-based high density cell culture devices, in preparation for *J. Immunol. Met.*,

R.C. Blake II, N. Omura, I.A. Darwish, A.M. Kriegel, S. Eackie, and D.A. Blake (2002) Monoclonal antibodies that bind to low- and high-molecular weight ligands with positive cooperativity, in preparation for *Science*.

D.A. Blake, R.M. Jones, R.C. Blake II, A.R. Pavlov, I.A. Darwish, and H. Yu (2001) "Antibody-based sensors for heavy metal ions", *Biosens. Bioelectron.*, in press.

Presentations

J.B. Delehanty, M. Khosraviani, H. Yu, and D.A. Blake (2000) "Probing the nature of the binding interaction between a Pb(II)-specific monoclonal antibody and its Pb(II)-chelate antigen". Experimental Biology 2000. April 15-18, San Diego, CA.

A.M. Kriegel, H. Yu, I.A. Darwish, R. Srinivasan, and D.A. Blake (2000) Production of mouse and rat monoclonal antibodies and stability of hybridoma cultures in membrane-based high density cell culture devices. 9th Annual Scientific Retreat, Tulane Interdisciplinary Graduate Program in Molecular and Cellular Biology. Oct. 21-22, Covington, LA.

A.M. Kriegel, R.M. Jones, I.A. Darwish, and D.A. Blake (2001) Anti-chelate monoclonal antibodies that exhibit unusual binding characteristics. 13th Annual Tulane Health Sciences Research Day, April 25-26, New Orleans, LA.

Intellectual Development

1. **Student Names:** James B. Delehanty and Alison M. Kriegel (graduate students in the Interdisciplinary Graduate Program in Molecular and Cellular Biology)
2. **Period of funding:** Delehanty, 5/29/00-6/30/01(received Ph.D. 6/25/01); Kriegel, April 2001-present
3. **Brief description of duties and responsibilities:** Delehanty cloned, sequenced, and expressed a recombinant antibody that recognized chelated complexes of Pb. Kriegel prepared large quantities of monoclonal antibodies for further development work; prepared proteolytic fragments of several monoclonal antibodies, and determined the binding characteristics of anti-chelate monoclonal antibodies

Useable Environmental Technologies

1. **Title of technology product:** Immunosensor for deployment in AUV; Recombinant antibodies for environmental analysis
2. **Description of technology product:** This antibody-based biosensor will be able to automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy (EARS);
3. **Utility/benefit/ROI/payoff of technology product:** A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters.
4. **Timeline (demonstration, validation, completion, etc.):** An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has already been established on the KinExA 3000 research instrument. Performance of the prototype assay in Mississippi River water will be assessed in the next 3 months. Transfer of the assay to the immunosensor will begin when Sapidyne completes the assembly of the instrument.

5. **Partners (academia, industry, labs/centers, federal agency, etc.):** Sapidyne Instruments (Boise, ID) is constructing the immunosensor and our laboratory is working closely with them to coordinate the development of biological reagents with the development of the instrument. The Blake laboratories also have strong ties with Dr. Fran Ligler's laboratory at the Naval Research Laboratory in Washington D.C. James Delehanty, who recently received his Ph.D. in Diane Blake's laboratory, is now an NRC fellow in Dr. Ligler's laboratory.
6. **Patents (applied for and issued):** J.B. Delehanty and D.A. Blake "Recombinant antibodies that bind to metal-chelate complexes", provisional patent application filed 3/29/2001.

Effects of Estrogens and Endocrine disrupters on Suppression of Apoptosis in normal and neoplastic Breast epithelial cells

Principal Investigator: Matthew E. Burow, Ph.D.
Research Assistant Professor
Center for Bioenvironmental Research
Department of Pharmacology
Tulane University

Co-Investigator(s): John A. McLachlan, Ph.D.
Professor and Director
Center for Bioenvironmental Research
Department of Pharmacology
Tulane University

Reporting Period: May 1999 – April 2001

Primary Objectives of Research Activities

The major goals of this project were to 1) develop technologies and methods to identify relevant organochlorine chemicals and dietary flavonoids that exert effects on estrogen responsive tissues and cell survival pathways, and 2) identify mechanism by which selected environmental agents would subvert the estrogen and cell survival signaling pathways thereby leading to potential developmental defects and or disease states (i.e cancer).

Progress Made to Achieve these Objectives

During the funded period we have identified a role for specific signaling pathways including the mitogen-activated protein kinase pathway (MAPK) functioning through AP-1 mediated transcription as a critical component of the estrogen mediated cell survival signaling pathway. The ability of estrogenic chemicals (estradiol, DES, DDT) to exert effects on cell survival pathways of breast carcinoma cells required an intact ERK-MAPK-pathway (1,4). In contrast anti-estrogens such as tamoxifen or ICI 182,780 as well as dietary flavonoid anti-estrogens exert negative effects on cell survival and ER activity through the JNK and p38 pathways (2-4,7). This understanding of the basic mechanisms of cell survival signaling through ER, AP-1 and MAPKs allowed us to develop in vivo screening technologies for AP-1 activating chemicals using stably transfected human endometrial and human embryonic kidney cell lines. These cell systems have allowed us to examine the ability of selected chemicals to activate AP-1 and related signaling pathway (Fos, Jun, Creb, Elk, Chop) through ER-dependent and independent mechanisms (5,6). The ability of environmental agents to regulate cell signaling and AP-1 pathways in an ER-independent mechanism forces us to expand our search for relevant endocrine disruption chemicals to include those outside the realm of estrogenic compounds.

Major Accomplishments

- Identified a role for MAPK signaling in conjunction with Bcl-2 expression in estrogen mediated cell survival signaling in breast carcinoma cells.
(publication # 4)
- Used relevant estrogen responsive reporter technologies to screen flavonoid

phytochemicals for estrogenic and anti-estrogenic activities towards MCF-7 breast carcinoma cells (publications #2,3)

- Identified a role for JNK and p38 MAPKs in signaling by flavonoid phytochemicals in the regulation of ER-mediated gene expression and proliferation of breast carcinoma cells (Publication # 2, 7)
- Identifies those flavonoid phytochemicals that demonstrate the ability to induce apoptosis or programmed cell death in human breast carcinoma cells (publication #8)
- Correlated relative estrogen receptor alpha and beta expression and signaling with apoptotic sensitivity and resistant among breast cancer cell variants (manuscript #1)
- Developed an *in vivo* mammalian cell culture assay to examine environmental relevant organochlorine molecules for estrogen receptor dependent and ER-independent activity toward cell signaling via mitogen-activated protein kinase (MAPK)-mediated activation protein 1 (AP-1) transcription. (publications # 4,5)
- Identified an role for organochlorine pesticides and flavonoid phytochemicals signaling to AP-1 via ER-independent mechanisms (publications #5-7).

Publications

Burow, M.E., Weldon, C.B., Chiang, T-C., Tang, Y., Collins-Burow B.M., Rolfe, K., Li, S., McLachlan, J.A., Beckman, B.S. Differences in protein kinase C and estrogen receptor α , β expression and signaling correlate with apoptotic sensitivity of MCF-7 breast cancer cell variants. *Int. J. Oncol.* 16: 1179-1187, (2000).

Collins-Burow, B.M., **Burow, M.E.,** Duong, B.N., McLachlan, J.A. Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and -independent mechanisms. *Nutrition and Cancer.* 38(2), 229-244 (2000).

Burow, M.E., Boue, S.B., Collins-Burow, B.M., Melnik, L.I., Duong, B.N., Li, S.F., Wiese, T., Cleavland, E., McLachlan J.A. Phytochemical glyceollins, isolated from soy, mediate anti-hormonal effects through estrogen receptor alpha and beta. *J. Clin. Endocrinol. and Metabolism* 86(4), 1750-1758, (2001).

Burow, M.E., Weldon, C.B., Tang Y., McLachlan, J.A., Beckman, B.S. Oestrogen-mediated suppression of TNF-induced apoptosis in MCF-7 cells: subversion of Bcl-2 by anti-oestrogens. *J. Steroid Biochem. & Mol. Biol.* 78(5): 409-418, (2001).

Frigo, D.E., **Burow, M.E.,** Mitchell, K.A., Chiang, T-C., McLachlan, J.A. DDT and its metabolites alter gene expression in human uterine cell lines through ER-independent mechanisms. Submitted to *Environmental Health Perspectives* (2001).

Burow, M.E., Collins-Burow, B.M., Frigo, D.E., Weldon, C.B., Elliot, S., Alam, J., McLachlan, J.A. Antiestrogenic activity of flavonoid phytochemicals mediated via c-jun N-terminal protein kinase and p38, Mitogen-activated protein kinase pathways. Isoform specific antagonism of estrogen receptor alpha. In preparation for submission to *Endocrinology*.

Collins-Burow, B.M., **Burow, M.E.**, Weldon, C.B., McLachlan, J.A. Induction of apoptosis by anti-estrogenic phytochemicals in breast carcinoma cells. Manuscript in preparation for submission.

Presentations

Burow, Matthew E. Bridgette M. Collins-Burow, Daniel E. Frigo, Christopher B. Weldon, Jawed Alam', and John A. McLachlan. Gordon Research Conference, Hormonal Carcinogenesis, 1999. Antiestrogenic Activity of Flavonoid Phytochemicals Mediated via c-Jun N-terminal Protein Kinase and p38 Mitogen Activated Protein Kinase Pathways. Tilton, New Hampshire

Collins-Burow, Bridgette M., Matthew E. Burow, Steve Boue, Lilia I. Melnik, Bich N. Duong, Tom Weiss, Ed Cleavland, John A. McLachlan Phytochemical glyceollins, isolated from soy, mediate anti-hormonal effects through estrogen receptor alpha and beta. Gordon Research Conference, Hormonal Carcinogenesis, 1999 Tilton, New Hampshire

Frigo, Daniel E., Peter M. Vonier, Matthew E. Burow, and John McLachlan. Structural Necessities for Estrogen Receptor Binding. 1999 Tulane Molecular and Cellular Biology Retreat.

Frigo, Daniel E., Matthew E. Burow, Kamron A. Mitchell, Steven Elliott, and John A. McLachlan. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 2000 Environmental Endocrine Disruptors Gordon Conference. June 20, 2000 – Plymouth, New Hampshire

Frigo, Daniel E., Matthew E. Burow, Kamron A. Mitchell, Steven Elliott, and John A. McLachlan. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. e.hormone 2000 Conference. October 16, 2000 – New Orleans, LA

Frigo, Daniel E., Matthew E. Burow, Kamron A. Mitchell, Steven Elliott, and John A. McLachlan. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 2001 AACR Annual Meeting. March 28, 2001 – New Orleans, LA

Frigo, Daniel E., Matthew E. Burow, Kamron A. Mitchell, Steven Elliott, and John A. McLachlan. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 2001 Tulane Health Sciences Research Days.

Frigo, Daniel E., Matthew E. Burow, Kamron A. Mitchell, Steven Elliott, and John A. McLachlan. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 2001 Environmental Signals and Sensors Center for Disease Control Meeting.

Frigo, Daniel E., Matthew E. Burow, Kamron A. Mitchell, Steven Elliott, and John A. McLachlan. The Effects of DDT and its Metabolites on AP-1 Activity: ER Dependent and Independent Mechanisms. 2001 Hormonal Carcinogenesis Gordon Conference. July 10, 2001 – Meriden, New Hampshire

Frigo, Daniel E., Matthew E. Burow, Kamron A. Mitchell, Tung-Chin Chiang, and John A. McLachlan. The Effects of DDT and its Metabolites on AP-1 Activity: Mechanisms of Environmental Signaling. e.hormone 2001 Conference. October 18, 2001 – New Orleans, LA

Intellectual Development

1. **Student Name(s):** Frigo, Daniel E. (Graduate Student 1999-present)
Mitchell, Kameron A. (MS, Public Health, Capstone research project 2000)
Collins-Burow, Bridgette M. (Medical Student research 1999, 2000)
2. **Funding Period:** 1999 - 2000
3. **Duties and Responsibilities:** Dan and Kameron - demonstrated that a select environmental compounds (with hormone activity) can stimulate the AP¹ transcription factor through estrogen receptor (dependent & independent). Bridgette – determined the estrogenicity of phytochemicals in breast epithelial compounds.

Useable Technologies

During the funding period in vivo cell culture models have been established for the examination of cell signaling pathways activation by relevant environmental contaminants. The cell systems can be utilized to screen extracts, mixtures of individual chemicals for activity on known cellular events involved in environmental toxicant responses. This type of screening would provide information as to potential deleterious effects of certain environmental chemicals or methodologies to classify these chemicals based upon unique cell-signaling profiles.

Epithelial Signaling as a Mechanism of Lung Injury to Inhaled Toxicant

Principal Investigator: Mitchell Friedman, M.D.
Edward G. Schlieder Educational Foundation
Professor of Pulmonary Diseases and Chief
Section of Pulmonary Diseases
Critical Care and Environmental Medicine
Tulane University School of Medicine

Co-Investigators: Arnold Brody, Ph.D.
Professor of Pathology and Laboratory Medicine
Tulane University School of Medicine

Joseph Lasky, M.D.
Associate Professor of Medicine
Section of Pulmonary Diseases
Critical Care and Environmental Medicine
Tulane University School of Medicine

Reporting Period: August 1999 – July 2000

Summary of Progress

Primary Objectives of Research Project

The primary objectives of this research project was to study how signals on cellular and molecular level can be utilized for assessment of human health and development of biosensors for assessments of toxicity and risk. Furthermore, the objectives were also based on the fact that the lung is the major site of toxicity for inhaled environmental Toxicant and that a "common final pathway" for the lung's response to environmental Toxicant is a fibroproliferative process. However, the precise molecular signaling mechanisms through which inhaled Toxicant cause fibroproliferative lung injury is not known. Since there are numerous potential mediators that can be expressed during disease development in the lung, it is essential that studies focus on selected molecules that could be key. We proposed to focus on two important signaling molecules (TNF- α and TGF- β 1) and their signal transduction pathways. Exposure to silica and/or asbestos induces lung inflammation, which may progress to fibrosis. Murine silica and/or asbestos challenge induce pathophysiological changes similar to the human disease states. The final theme of our cluster was the use of in vivo mouse models and in vitro macrophage cell models to study mechanisms of lung injury {leading to fibroproliferation, using silica and asbestos as models of inhaled Toxicant. These studies will help elucidate the underlying basic biological mechanisms of human disease with a particular focus on the lung.

The specific objectives of the proposal was:

In-Vivo Studies:

- (1) Use a mouse model in which we have transduced TGF- β by adenovirus vector into the lungs of the TNF- α RKO mice, to establish whether or not latent or active TGF- β induces fibroproliferative lung disease in the fibrogenic-resistant mice;
- (2) Expose the virally transduced animals to inhaled asbestos and establish TGF- β expression as a mediator of cell proliferation;

In-Vitro Studies:

- (3) Examine the differences in stimulation of the NF- κ B signal transduction cascade by silica in RAW 264.7 and IC-21 murine macrophage cell lines in relation to TNF α transcriptional activation; and
- (4) Identify differences in signal transduction pathways underlying silica-induced AP-1 activation in RAW 264.7 and IC-21 murine macrophage cell lines in relation to TNF α transcriptional activation.

Progress Made to Achieve These Objectives

In-Vivo Studies:

In 1999, we published a paper that showed that TNF- α receptor knock out (TNF- α RKO) mice are protected from the well-known fibrogenic effects of silica dust (Ortiz et al, 1999). This paper also was important because it demonstrated that the higher molecular weight (P75) receptor is key to the signal transduction pathways necessary for the biological effects of TNF- α after silica or bleomycin exposure. We have also published two additional papers. In one we show that TGF- β_1 is expressed at sites of asbestos-induced lung injury (Liu and Brody, 2001). This paper identified the list of peptide growth factors that we had proposed were produced by the bronchiolar-alveolar epithelium during the development of fibrogenic lesions. The second paper used a non-replicating adenovirus vector to overexpress TGF- β_1 in the lungs of normal mice and the TNF- α RKO mice (Liu, et al., 2001). This latter publication is key for two reasons: 1) TNF- α RKO mice exhibit reduced expression of TGF- α , PDGF and TGF- β_1 while TNF- α expression remains high after lung injury. This paper also shows that replacing TGF- β_1 expression is sufficient to induce interstitial inflammation and fibrogenesis. Prior to this finding, there were no data upon which to predict whether or not the fibrogenic-resistant TNF- α RKO mice would develop interstitial lung disease.

In-Vitro Studies:

To further study the upstream pathways leading to TNF α transcriptional activation, and dissect the mechanisms underlying the different sensitivity to silica-induced fibrosis among the various murine strains, we needed to identify an appropriate *in vitro* model. Therefore, we screened murine macrophage-derived cell lines for their response to silica-induced TNF α secretion. We have identified two cell lines, one that exhibits enhanced TNF α production in response to silica (RAW 264.7), and another cell line which does not produce TNF α in response to silica (IC-21). We hypothesized that differences in TNF α transcriptional induction by silica in the murine macrophage cell lines IC-21 and RAW 264.7, are mediated by differential activation of signal transduction pathways underlying AP-1 and NF- κ B activation, resulting in differences in gene products involved in lung inflammation and fibrosis. In the in-vitro studies, we found that the two murine macrophage-derived cell lines differed not only in their TNF α responses but also in

their apoptotic responses to silica *in vitro*. RAW 264.7 macrophages exhibit enhanced TNF α production and NF- κ B activation in response to silica. In contrast, IC-21 macrophages do not produce TNF α in response to silica and do not induce NF- κ B. However, both IC-21 and RAW 264.7 macrophages are able to phagocytize silica particles and induce TNF α production and NF- κ B activation in response to lipopolysaccharide (LPS). We therefore employed this novel paradigm to further examine relationships between silica-induced apoptosis, TNF α production, and NF- κ B activation. The RAW 264.7 macrophage cell line was more sensitive, and the IC-21 macrophage cell line more tolerant to silica exposure (0.2 or 1 mg/ml for 6 hours) as evidenced by significantly higher apoptotic responses in RAW 264.7 ($P < 0.05$). RAW 264.7 macrophages exhibited enhanced TNF α production and NF- κ B activation in response to silica, whereas IC-21 macrophages did not produce TNF α in response to silica and did not induce NF- κ B nuclear binding. Inhibition of NF- κ B in RAW 264.7 cells with BAY11-7082 significantly increased apoptosis, while inhibiting TNF α release. In addition, TNF α and NF- κ B activation but not apoptosis were induced by LPS in both cell lines, and NF- κ B inhibition inhibited LPS-induced TNF α release. These data suggest that TNF α induction is dependent on NF- κ B activation in both cell lines. However, silica can induce apoptosis in murine macrophages, independently of TNF stimulation as in IC-21 macrophages. Furthermore in RAW 264.7 macrophages, NF- κ B activation may play dual roles, both pro- and anti-apoptotic during silica injury.

Major Accomplishments

In summary, progress during the grant period has been substantial, with several peer-reviewed publications and abstracts presented at national conferences.

The most important component of the in-vivo studies is the demonstration that there is a significant role of TGF- β_1 in the pathogenesis of interstitial pulmonary fibrosis, secondary to inhaled toxicants.

- We showed that TGF- β_1 is diminished in the lungs of mice that develop a reduced disease response and that transient re-constitution of TGF- β_1 expression returned a fully developed fibroproliferative disease process to the TNF- α RKO mice.
- The adenoviral vector now can be titrated and the degree of disease manipulated such that we can propose to develop an anti-sense cRNA that will block TGF- β_1 and TNF- α translation.
- NF- κ B activation is essential to TNF α induction following silica exposure and plays an anti-apoptotic role in macrophages. Silica-induced activation of NF- κ B cells may protect the cells from both TNF α - and silica-induced injury.
- Silica-exposed IC-21 macrophages did not release TNF α , and did not induce NF- κ B DNA-binding. The discrepancy in the cellular response between these cells and cells sensitive to silica appears to be specific to silica, since LPS stimulation induced TNF α release and NF- κ B DNA binding in both cell lines.

These observations further imply that the studies looking at mechanisms of injury for inhaled toxicant such as silica should not target one signaling molecule, but rather a signaling pathway.

Publications, Manuscripts and Abstracts

Papers:

Ortiz, L. A., Lasky, J., Lungarella, G., Cavarra, E., Martorana, P., Banks, W. A., Peschon, J. J., Schmidts, H. -L., Brody, A. R., and Friedman, M. Upregulation of the p75 but not the p55 TNF- α Receptor mRNA after silica and bleomycin exposure and protection from lung injury in double receptor knockout mice. *Am. J. Respir. Cell Mol. Biol.* 20:825-833, 1999.

Brass, D., Hoyle, G. W., Poovey, H.G., Liu, J. -Y., and Brody, A.R. Reduced TNF- α and TGF- β 1 expression in the lungs of inbred mice that fail to develop fibroproliferative lesions consequent to asbestos exposure. *Am. J. Pathol.* 154:853-862, 1999.

Ortiz, LA, Lasky, J, Banks W, Peschon, JJ, Friedman, M. Upregulation of the p75 but not the p55 TNF receptor mRNA during silica and bleomycin-induced lung injury in mice. *Am. J. Respir. Cell Mol. Biol.* 20:825-833, 1999.

Liu, J. -Y. and Brody, A. R. Increased TGF- β 1 in the lungs of asbestos-exposed rats and mice: Reduced expression in TNF- α receptor knockout mice. *J. Environ. Pathol. Toxicol. Oncol.* 20(2):97-108, 2001.

Liu, J.-Y., Sime, P. J., Wu, T., Warshamana, G. S., Pociask, D., Tsai, S.-Y., and Brody, A. R. Transforming Growth Factor- β 1 overexpression in Tumor Necrosis Factor- α receptor knockout mice induces fibroproliferative lung disease. *Am. J. Respir. Cell Mol. Biol.* 25:3-7, 2001.

Warshamana, G. S., Corti, M., and Brody, A. R. TNF- α , PDGF, and TGF- β 1 expression by primary mouse lung bronchiolar-alveolar epithelial and mesenchymal cells: TNF- α , induces TGF- β 1. *Exp. Mol. Pathol.* 71:13-33, 2001.

Lasky, J.A., M. Friedman, Y. Zhuo, J-Y Liu, H. Poovey, A.R. Brody. Platelet-derived growth factor receptors are essential in the development of asbestos-induced fibrosis. *CHEST* 120:S61, 2001.

Abstracts:

Lasky, J.A., E. Gozal, D.M. Brass, H. Lu, M. Friedman, A.R., Brody. A PDGF-sensitive tyrosine kinase inhibitor significantly blocks asbestos-induced lung fibroblast proliferation. *Am. Rev. Respir. Crit. Care Med.* 1999; 159:A72.

Gozal, E., M. Friedman, X. Zou, M.A. Reyes, L. Ortiz. Absence of both p55 and p75 TNF α receptors is required to prevent NF-KB activation in bleomycin (BLM)-induced lung fibrosis. *Am. Rev. Respir. Crit. Care Med.* 1999; 159:A928.

Ortiz, L., J. Lasky, M. Reyes, G. Lungarella, P. Martorana, E. Cavarra, A. Brody, A. Pardo, M., Selman, M., Friedman. Enhanced expression of interstitial collagenase in the lung of individual tumor necrosis factor (TNF) receptor deficient mice. *Am. Rev. Respir. Crit. Care Med.* 1999; 159:A71.

Ortiz, L., E. Gozal, Z. Xiang, M. Reyes, M. Friedman. Individual (p55 or p75) TNF receptors activate I κ B kinase and promote NF-kB activation in bleomycin (BLM)-induced lung fibrosis. *Eur. Respir. J.* 1999; 14:A660.

Liu J, Hoyle G, Brass D, and Brody A. Expression of TGF- β 1 at sites of lung injury in normal rats and TNF- α receptor knockout mice. *Am J Respir Crit Care Med.* 159:A929, 1999.

Gozal, E., L. Ortiz, X. Zou, M. Reyes, M. Burow, J. Lasky, and M. Friedman. Role of TNF- α and NF-kB in silica-induced apoptosis of raw 264.7 and IC-21 murine macrophage cell lines. *Am. Rev. Respir. Crit. Care Med.* 2000; 161:A666.

Ortiz, L., J. Lasky, E. Gozal, G. Lungarella, P. Martorana, E. Cavarra, A. Pardo, M. Friedman, and M. Selman. Altered MMP-13/TIMP-1 RNA expression and decreased AP-1, but not NF-kB, activation characterize TNF receptor knockout mice resistance to silica. *Am. Rev. Respir. Crit. Care Med.* 2000; 161:A481.

Liu J-Y, Pociask DA, Warshamana GS, and A. Brody AR. Induction of fibrosis by adenovirus-mediated expression of active TGF- β 1 in TNF- α receptor knockout mice (TNF- α RKO). *Am J Respir Crit Care Med.* 161:A465, 2000.

Warshamana GS, Pociask DA, Liu J-Y, Fisher K, and Brody AR. Titration of adenovirus-mediated expression of active TGF- β 1 in two strains of mice developing fibroproliferative lung disease. *Am J Respir Crit Care Med.* 161:A667, 2000.

Presentations

All listed abstracts above have been presented at the annual meetings of the American Thoracic Society.

Intellectual Development: None

Useable Environmental Technologies: None.

Ecological Remediation: Resurrection of an Extinct Species of Frog from Puerto Rico

Principal Investigator: Scott F. Michael, Ph.D.
Department of Tropical Medicine
School of Public Health and Tropical Medicine
Tulane University Health Sciences Center

Reporting Period: August 1999 – April 2001

Primary Objectives of Research Activities

- 1) Determine if frozen sperm remain competent to direct development of enucleated eggs from the same species.
- 2) Determine if frozen sperm are able to direct development of enucleated eggs from a closely related species.
- 3) Determine if frozen sperm from an extinct species are able to direct development of enucleated eggs from a closely related extant species.

Progress Made to Achieve these Objectives

An inability to easily initiate egg activation and development in unfertilized eggs has been a major roadblock for this project. To overcome this, large numbers of eggs have been needed for a variety of alternate experimental plans. We were successful in obtaining small numbers of eggs from naturally breeding animals by separating the males and females before fertilization. Larger numbers of eggs could be obtained by artificially induced ovulation, as is done with many other amphibian species. However, injections of human chorionic gonadotropin do not cause ovulation in *E. coqui*, as they do in most other species. One of our successes has been to identify a synthetic leutinizing hormone releasing hormone that produces consistent ovulation and egg deposition in *E. coqui*. This has led to the ability to test a wide variety of egg activation strategies, some of which are candidates for consistent use in this species.

None of the commonly used amphibian egg activation techniques appear to work for *E. coqui* eggs. However, we have preliminary evidence that both the use of peptides that mimic sperm surface proteins as well as the use of UV irradiated sperm can be used to activate *E. coqui* eggs while bypassing normal fertilization. During this work we have developed methods for sperm cryopreservation and sperm irradiation, as well as egg irradiation using gamma radiation to inactivate the female pronucleus.

The result that we have obtained that most closely achieves the objectives of the original proposal is the finding that long-term frozen sperm from *E. coqui* as well as several other species remains competent to initiate nuclear decondensation in cytoplasmic extracts prepared from *E. coqui* eggs. This result indicates that long-term frozen sperm from

these frogs is largely undamaged, capable of initiating development, and that the initial steps at least are largely species independent. This result, in and of itself, gives significant support to the hypothesis that methods can be developed to resurrect extinct *Eleutherodactylus* species.

Major Accomplishments

- Artificial induction of ovulation for the production of large numbers of eggs.
- Demonstration of nuclear decondensation from long-term frozen sperm.
- Sperm cryopreservation.
- In vitro fertilization and generation of viable frogs.
- Preliminary observations of induced egg activation.

Publications, Manuscripts, Abstracts

Vincent, S., Carlson, J. and Michael, S.F. Artificially induced ovulation and egg deposition in the Anuran *Eleutherodactylus coqui*. In Preparation for: Journal of Comparative Physiology A.

Jones, C. and Michael, S.F. Cryopreservation of sperm from the Anuran *Eleutherodactylus coqui*. In Preparation for: Journal of Cryobiology.

Presentations

Michael, S.F., West Indian *Eleutherodactylus* Frogs: Acoustic Communication and Conservation Issues. University of New Orleans. Department of Biology Seminar Series. September 11, 1999

Intellectual Development

1. **Student Name:** Cecily Jones, Xavier SPRITE student
2. **Period of Funding:** 5/00-12/00
3. **Duties and Responsibilities:** Sperm cryopreservation studies.

1. **Student Name:** Shawn Vincent, Loyola undergraduate research assistant
2. **Period of Funding:** 5/00-4/01 (Tulane EEOB graduate student 5/01-present)
3. **Duties:** Induced ovulation, in vitro fertilization and egg activation studies.

1. **Student Name:** John Carlson, Tulane Tropical Medicine graduate student.
2. **Period of Funding:** 8/99-present
3. **Duties:** In vitro fertilization, egg activation and nuclear decondensation studies

4. **Student Name:** Sebastian Lourido, Tulane undergraduate research assistant
5. **Period of Funding:** 9/00-present
6. **Duties:** In vitro fertilization and egg activation studies.

Useable Technologies: None

Field Experiment to Characterize Habitat Preferences of Key Mosquito Species on the North Shore of Lake Pontchartrain, Louisiana

Principal Investigator: Dawn Wesson, Ph.D.
Associate Professor
Medical/Molecular Entomology
School of Public Health and Tropical Medicine
Tulane University Health Sciences Center

Co-Investigators: Richard Campanella, MS
Environmental Analyst/Remote Sensing-
GIS Specialist
Center for Bioenvironmental Research
at Tulane and Xavier Universities

Reporting Period: May 1999 – April 2001

Primary Objectives of Research Activities

The objective of this field experiment is to characterize the habitat preferences, spatial patterns, and temporal trends in mosquito species (captured through traps and through landing-biting) at an array of sites in the semi-rural North Shore of Lake Pontchartrain, Louisiana.

We are targeting *Culiseta melanura*, a species which has long been implicated as a maintenance vector of the Eastern Equine Encephalitis (EEE) cycle throughout the United States. This ornithophilic mosquito utilizes unique breeding and resting habitats (such as low-lying woodlands featuring broad-leafed trees) which have been well characterized in the northeastern United States, from Massachusetts to Maryland. However, *Culiseta melanura* habitats have not been well examined in Louisiana, a state that had 97 confirmed EEE horse cases in 1999.

In addition to *Culiseta melanura*, we will also analyze a wide number of other species, many of which are of public-health and scientific interest. Species captured to date include *Aedes vexans*, *Anopheles atropos*, *An. crucians*, *An. punctipennis*, *An. quadrimaculatus*, *Culex erraticus*, *Cx. nigripalpus*, *Cx. quinquefasciatus*, *Cx. restuans*, *Cx. salinarius*, *Cx. territans*, *Culiseta inornata*, *Ochlerotatus canadensis*, *Oc. cinereus*, *Oc. dupreei*, *Oc. infirmatus*, *Oc. sollicitans*, *Oc. taeniorhynchus*, *Oc. triseriatus*, *Oc. trivittatus*, *Uranotaenia sapphirina* and *Ur. lowii*.

Progress Made to Achieve these Objectives

The first phase of the experiment is complete. This phase consisted of the establishment of 7 sites (selected through the use of remote sensing and GIS as seven distinct ecological/geographical environments, in terms of land cover, vegetation, and topography) at which we prepared and established 4 fiber-pot traps oriented in the cardinal directions, a data logger (recording temperature and relative humidity), and a rain gauge. These sites were visited by 3-6 project participants 23 times between

February 21 and June 8, 2001. Mosquitoes were removed from the traps with an aspirator, then a 5-minute landing collection was conducted at each site. Laboratory staff then analyzed each specimen and stored the results in a database. These data are currently being analyzed.

The second phase of this field experiment comprises the re-establishment of a larger and more representative array of sites, totaling 15 instead of 7 and arranged as a regular 3 x 5 grid imposed over a quarter-mile area of maximum ecological/geographical diversity. This grid design represents an inductive approach to revealing patterns of habitat preference, as opposed to the deductive approach of the 7 habitat-driven points. We established this grid (using GPS and machetes) in May 2001 and began collections in mid-June 2001 at a pace of twice a week, involving 3-5 people each time. A typical field trip takes 4 hours to complete.

Researchers at the laboratory follow up each field collection with an analysis of each mosquito, determining its species, gender; if gravid and bloodfed. These data are stored in a database and will be analyzed later through GIS.

Major Accomplishments

- Collection of a vast and detailed dataset of mosquito species and characteristics, along with corresponding ecological/geographical/climatic data, at 7 sites. These data are currently being analyzed.
- Design, selection, and establishment of a grid of 15 points over a quarter-mile area (through extremely dense vegetation) and setting up of data loggers, traps, and rain gauges. This field experiment will prove to be a valuable asset, as we assemble and analyze a legacy of carefully collected data over time.
- Establishment of sampling procedures and schedule of participants to tend to these sites.
- Experimentation with using commercially available fiber pots for the purpose of trapping mosquitoes.

Publications, Manuscripts, Abstracts

We plan to publish results as we proceed with data analysis.

Presentations

We plan to present findings on a wide range of topics as we proceed with data analysis.

Intellectual Development

1. **Student Name(s):** Bethany Peel, Bryan Shelby, Clara Ocampo, Summer Nguyen, Craig Conard, Maria Morales, Andrea Von Burg.
2. **Funding Period:** May 1, 1999 – April 30, 2001
3. **Duties and Responsibilities:** All students have participated in the following activities: GPS location of sites using handheld navigation system, establishment of

collection sites, collection and processing of live mosquitoes, identification of mosquitoes, data analysis.

Useable Environmental Technologies

The fiber pots used as resting boxes for this study have been described previously for this work, but they are not widely used in Louisiana for mosquito sampling. Although relatively "low-tech", they may be especially effective for collecting *Culiseta melanura*, thought to be an important maintenance vector of EEE. *Culiseta melanura* is otherwise very difficult to collect with traditional methods and so there is little information available as to its temporal or spatial distribution in the state. Following the first season of collections from North Shore sites, information on the effectiveness of this technique will be presented at annual meetings of both the Louisiana Mosquito Control Association (for in-state information dissemination) and the American Society of Tropical Medicine and Hygiene (for regional and national dissemination).

Human Health Applications

Principal Investigator: Valerie Wilson, Ph.D.
Deputy Director and Professor
Center for Bioenvironmental Research
at Tulane and Xavier

Reporting Period: May 2000 –April 2001

Primary Objectives of Research Activities

(1) Expand a population-based study of uterine fibroids incidence in women; (2) develop collaborations with other researchers; (3) develop information for the public.

Progress Made to Achieve these Objectives

(1) Demographic information, symptomatology, and fibroid pathology data were abstracted from patients undergoing treatment for uterine fibroids at the Medical Center of Louisiana at New Orleans and a private physician office in Metairie. (2) Preliminary contacts were made with other investigators conducting similar studies on uterine fibroids and other estrogen-related conditions. (3) Using MEDLINE, peer-reviewed journal articles with synonyms of the keywords “uterine fibroids” and “environment” are identified and are used to construct a literature review.

Major Accomplishments

(1) We abstracted data from 426 subjects. Two studies were attempted using the abstracted data. The purpose of the first study was to provide a description of the risk factors for uterine fibroids in a population of New Orleans women. Of the 426 eligible subjects, 368 had sufficient data for analysis. We analyzed these subjects based on age, race, BMI, age at menarche, and age at menarche by 5-year age category. The patient population in our cohort is similar to populations described in other studies on uterine fibroids with respect to age, race, obesity, high prevalence of hysterectomy, and age at menarche. We then evaluated if any of these factors might be correlated to increased fibroid number among women who underwent hysterectomy only. Selection criteria for this study were: 25-64 year old; pre-menopausal; uterine fibroids as the primary diagnosis; underwent hysterectomy between July 1995 to June 2000; and subjects lived in the Greater New Orleans area, as determined by zip code. We identified 360 potential women from our original cohort of 426. Of these, only 155 had sufficient information in their charts and met the inclusion criteria. After careful review of the chart information, none of the risk factors examined in Study 1 were significant predictors of multiple uterine fibroids, though BMI (a measure of obesity) was nearly significant. This study was inconclusive due, in part, to an inability to obtain a significant number of study subjects and not enough information in the medical charts from Charity hospital records. We conclude from these studies that New Orleans women with fibroids have a demographic and health profile similar to that of women in other cities, and that the decline in the age of menarche may implicate an environmental factor (yet to be defined) in the condition. (2) We described previously collaboration with a local physician that

specializes in laser treatment of fibroids. Because of limitations inherent in the Charity surgical records, our future research will focus on data from the private physician's office. In addition, during a recent symposium sponsored by the CBR, we tentatively established a partnership with the University of Puerto Rico to study the epidemic of premature thelarche (premature breast development in girls that occurs long before sexual maturity) cases that have occurred over the past 20 years. In a similar light, we established a partnership with one of our CBR Distinguished Scholars, Elizabeth "Buzzy" Guillette, PhD, who has studied the effects of pesticides on the health of young children in a heavily agricultural region near Ciudad Obregon, Mexico. We successfully secured funding for a pilot study at this site to examine if signs of premature puberty is seen in young girls who have previously been exposed to these pesticides. (3) Only seven peer-reviewed articles directly studied uterine fibroids and the possible role of environmental chemicals such as DDT, polychlorinated biphenyls, and other xenoestrogens in the pathogenesis of the condition. The link is tentative at best, and clearly more long-term, prospective approaches are necessary to elucidate this connection. (See Appendix 1 for an updated literature review of uterine leiomyoma, which includes a discussion on the link between the environment and uterine fibroids.)

SIGNIFICANCE: (1) The data that we have been able to collect from Charity Hospital records has been very useful to confirm existing data about occurrence, rate and demographics of uterine fibroids in the local population compared to US statistics. However, these data have limitations that do not allow us to retrieve the type of information that we need for future studies nor are the data in sufficient numbers to be useful. These limitations are due to the purpose for which the records exist originally – that is, surgically important information for safety during surgical procedures only. In other words, the pilot study revealed that because the data were not developed for research purposes, the research questions that we need to ask would require other sources to obtain that information (See (2) for the future sources of that information). (2) Because the private physicians' data records contain pre- and post-surgical care information in great detail, they should provide the necessary information to structure the research questions we wish to ask. In terms of other estrogen-dependent condition, with additional support, we hope to establish a long-term epidemiological study in Puerto Rico and/or Mexico that will assess the influence that man-made chemicals have had on early puberty and the role that this exposure may play in the development of benign uterine diseases. (3) Some risk factors have been identified as having an increased or decreased risk on fibroid development. While it would appear that these factors relate to estrogen in some way, they still fail to paint an accurate picture of fibroid risk. More long-term epidemiological studies are needed to determine how these risk factors fit together. Perhaps then we can develop a model that can predict a women's risk of developing fibroids, given the presence of certain risk factors.

Work Plan (next 12 months): (1) Little has been published on the risk factors associated with women who have multiple fibroids. Equally enigmatic is the prevalence of pregnancy following procedures that take exquisite care in decreasing post-operative blood loss and adhesion formation, which have been shown to decrease fertility. The surgical procedure used by the private physician is such a procedure. Toward that end,

we recently developed a more detailed chart abstraction form and a questionnaire that will be mailed to former patients of Dr. Moorehead. Using these tools, we hope to answer several questions about surgical complications, restoration of fertility and fibroid recurrence following this procedure. (2) Our uterine fibroid work will be focused on long-term care records, primarily from the private physician's medical practice. One of the lead researchers on premature thelarche in Puerto Rico will be giving a presentation this Fall at the CBR's environmental symposium. We hope to further explore our tentative partnership and actively seek funding to establish a long-term study in Puerto Rico. In addition, Dr. Guillette and our Field Epidemiologist, Craig J. Conard, will spend two weeks this summer studying the effects of pesticide exposure on premature sexual development in young girls. (3) We will continue to monitor the literature to ascertain environmental factors that posit a link to the development of fibroids.

Appendix 1.

Uterine Leiomyoma Literature Review

The basis of this project is to evaluate and/or propose various environmental factors that may be complicit in human health conditions. In order to evaluate the various environmental hypotheses that may exist, a CBR student scholar conducted a literature review three years ago. During the past year, we updated that literature review. Below is a synopsis of the peer review finding to date.

Introduction

While uterine leiomyomas (fibroids) are the leading cause of hysterectomies in the United States (Wilcox et al 1994), its etiology remains unclear. Recent research has focused on the role of estrogens in fibroid development. It has been well documented that fibroids grow in the presence of estrogen and shrink in the absence of estrogen. Since some natural and man-made chemicals have similar chemical structures to estrogen, they have been shown to cause endocrine responses in both animal and human studies. Thus scientists have begun to assess whether estrogens in the diet and exposures to these mimicking chemicals are causative factors in the development of uterine fibroids. The following is a literature review of the general aspects of uterine leiomyoma followed by a review of its epidemiology, with a specific focus on race and obesity. The last section examines the role that the environment plays in fibroid development.

Uterine Fibroid Overview

Uterine leiomyomas (fibroids) are benign neoplasms derived from the smooth (non-striated) muscle of the uterine wall. While fibroids may be found in any of the layers of the uterine wall (serosal, myometrial, and mucosal), most are commonly found within the myometrial layer. Fibroids are associated with a variety of symptoms including abnormal bleeding, pelvic pain, activity limitation, anemia, fatigue, urinary and bowel problems, miscarriage and infertility. The severity of symptoms usually depends on the size, position, and number of fibroids present.

True incidence of fibroids in the population is difficult to ascertain, for studies have placed the figure as low as 20%, and as high as 77%, in women over 30 years of age.

Most studies reporting the frequency of fibroids used clinical diagnoses and pathology reports to diagnose women with fibroids. It is unclear whether using these methods accurately approximates the true incidence of fibroids. One study using serial sectioning tripled the number of fibroids noted in routine pathology reports, reporting that the incidence of fibroids could actually be as high as 77% (Cramer and Patel 1991). This study may indicate that the incidence of fibroids has been underestimated using current diagnostic standards (ultrasound, clinical exam, pathology reports etc.)

Fibroid Risk Factors

Table 1 provides a good summary of the factors that reduce risk, that increase risk, and those factors that have an unclear role in fibroid development.

Reduce Risk	Increase Risk	Uncertain
• Multiparity • Smoking (current and ever) • Older age at last term pregnancy • Menopause • Breast feeding	• Age (35-54) • Race (African American) • Obesity (Body Mass Index) • Unopposed estrogen • Higher education • Family history • Younger age at menarche	• Oral Contraceptives • Endocrine disrupting chemicals

Table 1. Risk factors associated with uterine fibroid development.

The one theme that unifies these factors is estrogen, regardless of the positive or negative effect each factor has on leiomyoma development. For example, it has been hypothesized that smoking decreases fibroid risk by reducing the exposure of the myometrium to estrogens. Another example is menopause; the lack of circulating estrogen decreases leiomyoma size and may protect against further development of leiomyomas. The association between age and increased fibroid risk can be attributed to higher estrogen levels, for as women age, they are exposed to more unopposed estrogen, thereby increasing risk. Scientists hypothesize that the increased fibroid risk in obese women is related to an increase in circulating estrogen levels in these women as compared to non-obese women. This same mechanism holds true for women with a younger age at menarche; a woman experiences an increased risk the earlier she is exposed to estrogen. The uncertain factors lack convincing evidence to support how these factors affect estrogens effect.

The issue of race: Perhaps the most studied risk factor in leiomyoma development is race. While numerous studies have shown that African-American women are disproportionately affected, to date there has been no clearly established reason for this

difference. Most studies have placed the relative risk of African-American women developing fibroids as 2-3 times greater than white women (Wilcox et al. 1994, Marshall et al. 1995). African-American women tend to be younger at the time of surgery for fibroids than white women (42 years vs. 46 years) and have had over twice the number of hysterectomies with fibroids as the diagnosis (Kjerluff et al. 1993). Another study has identified that African-American women tend to be younger at their first birth of a child and older at their last birth as well as at the time of diagnosis than white women (Marshall et al. 1995). African-American patients are more likely to have both subserosal and submucosal leiomyomas. [Kjerluff, 1996 #68] Fibroids are also more likely to achieve a larger size at an earlier age in African-American women (Robbins et al. 1979). It has also been observed that African-Americans receive fewer routine gynecological exams (Thompson and Rock 97, Kjerluff 93). This might indicate that these women tend not to seek medical treatment until the fibroids are very large, at which time uterine sparing treatments may be too late and hysterectomy is required. In fact, it has been found that African-American women were more likely to have abdominal hysterectomy than white women to remove fibroids. As a result, African-American women were also more likely to experience complications in surgical or medical care for fibroids (Kjerluff et al. 1996).

Despite these findings, this does not answer the question of why African American women are disproportionately affected and what, if anything, can be done to prevent fibroids from occurring in this group. What is needed is a large, prospective, nation-wide study of women that compares the risk among various racial groups, not just white vs black. Perhaps other racial groups share a similar high fibroid risk, in which case other factors, like genetics, may have a more significant role than previously believed.

Fat distribution and fibroid risk: Some studies have noted obesity is positively associated with the development of uterine fibroids. While body mass index (BMI) has been chosen as the standard to assess obesity for most epidemiological studies, it remains unclear how accurate it is as an indicator of obesity (Solomon et al. 1997, Wellen et al. 1996, Baumgartner et al. 1995). BMI is calculated by dividing the individual's weight in kilograms by the square of his height in meters. According to the National Institutes of Health (NIH) BMI scale, a BMI of less than 25 is considered normal; a BMI between 25.1 and 29.9 is overweight; and a BMI greater than 30 is considered obese. Other obesity measurement tools include, but are not limited to, electrical impedance (a small amount of electricity is applied to a pinch of skin, the voltage is measured and compared with previously known standards to measure the amount of total body fat), sub-scapular skin-fold thickness (a small gauge is used to pinch the skin directly underneath the back shoulder bone), and densitometry (weighing a person underwater and using Archimedes' principle, which states that the volume of water displaced equals the volume of water the object displaced. This difference is the total body volume).

No study to date has analyzed which obesity indicator is most closely associated with uterine fibroid development. As expected, most fibroid studies have used BMI as their obesity indicator and have observed that BMI is significantly associated with uterine fibroid development (Marshall et al 1995). However, as some studies have shown, BMI

does not discern between the different types of body fat distribution, nor does it differentiate between body fat and lean body mass (Wellen et al. 1996, Baumgartner et al. 1995). To solve this problem, some researchers have used a composite of BMI and body fat distribution to examine fibroid risk. One such study investigated the etiological relationship between body fat (a composite of BMI and percent body fat measured by electrical impedance) and uterine leiomyomas and concluded that those with upper body fat distribution and occult obesity (defined as a BMI of less than 24 and percent body fat greater than 30%) were at the greatest risk of developing leiomyomas (Sato et al. 1998). Thus, it appears as if obesity increases risk of fibroid development. Scientists hypothesize that the increased risk of fibroids in obese women is related to increased circulating estrogen in obese women as compared to non-obese women. It is believed that fat cells sequester estrogen and once these cells are metabolized, they release estrogen. This increased concentration of estrogen in the blood may further stimulate fibroid development. This role is examined further in the section below.

Estrogen, the environment, and fibroid risk

It is commonly accepted that uterine fibroids increase in size during pre-menopause and regress post-menopausally. Studies have found that the incidence of fibroids was similar for postmenopausal women when compared to premenopausal women although postmenopausal fibroids were fewer and smaller than premenopausal ones (Cramer and Patel 1991). Other studies have reported increased number and over-expression of both estrogen and progesterone receptors in fibroid tissue when compared to normal myometrium (Sadan et al 1990, Englund et al 1998). One unpublished study (Walker et al) presented at the CBR "Estrogens in the Environment" conference in 1996 noted that leiomyoma tumor-derived cell lines of Eker rats express estrogen receptors and bind estrogen specifically. The authors also determined that growth *in vitro* was stimulated by estrogen and inhibited by anti-estrogens. Consequently, certain endocrine disrupting chemicals, especially those that have been found to mimic estrogen, may have an effect on fibroid growth. Like estrogen, these chemicals tend to accumulate in fat and once metabolized, may cause endocrine-like effects. It has been hypothesized that there is an apoptotic defect in leiomyoma cells (Walker et al 1998). Since estrogen has been linked to apoptosis in breast cancer and endometrial cells, this raises the question of what effect xenoestrogens would have on proliferation of leiomyoma cells, given that estrogen is implicated in proliferation but not regulation of apoptosis.

Recent research suggests that the link between fibroids and chemicals that mimic estrogen is tentative at best. Saxena et al found that DDT levels in the blood of women with uterine leiomyoma were three times higher than women without leiomyoma. They also found that leiomyoma tissue had significantly higher levels of DDT and its metabolites when compared to non-leiomyomatous tissue in the same individual. Studies found that alpha hexachlorocyclohexane (HCH) and cadmium were found in the urine of women with uterine fibroids (Gerhard and Runnebaum 1992). In another study, six of nine xenoestrogens (DES, genistein, coumestrol, naringenin, endosulfan- α , and endosulfan- β) indicated evidence of agonist activity in myometrial cells of the Eker rat in multiple assay, demonstrating the potential for an impact of xenoestrogens on both transformed and normal myometrial tissues (Hunter et al. 1999). It is important to note

that these results were obtained from mostly laboratory and animal studies. Clearly more epidemiological research is necessary to determine whether these effects are seen in human populations as well.

Conclusion

Some risk factors have been identified as having an increased or decreased risk on fibroid development. While it would appear that these factors relate to estrogen in some way, they still fail to paint an accurate picture of fibroid risk. More long-term epidemiological studies are needed to determine how these risk factors fit together. Perhaps then we can develop a model that can predict a women's risk of developing fibroids, given certain risk factors that are present in the women.

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Publications

None

Presentations

None

Intellectual Development

N/A

Useable Environmental Technologies

N/A

ECOSYSTEM MONITORING AND ASSESSMENT

Extraction and Detection of Selected Pharmaceuticals and Personal Care Products (PPCPs) in Coastal Areas of Southeastern Louisiana

Principal Investigator: Glen R. Boyd, Ph.D., P.E.
Assistant Professor
Civil and Environmental Engineering Department
Tulane University

Co-Investigator(s): Siddhartha Mitra, Ph.D.
Senior Research Scientist
Civil and Environmental Engineering Department
Tulane University

Reporting Period: May 2000 – April 2001

Primary Objectives of Research Activities

To develop a standard methodology for the efficient extraction of several PPCPs from Mississippi River and coastal Louisiana particulate and water samples.

Progress Made to Achieve these Objectives

Detailed laboratory investigations were conducted to develop methods for collecting, preparing, and quantitatively determining eleven representative pharmaceutical and personal care products (PPCP) and endocrine disrupting chemicals (EDCs) in water. The method consists of solid phase extraction and derivatization using gas chromatography/mass spectrometry (GC/MS) for quantitative analysis. The method can be applied to surface water (e.g., Mississippi River, Lake Pontchartrain) and drinking water samples.

Major Accomplishments

- We developed and documented standard operating procedures for extraction, quantification, and monitoring of eleven PPCPs and EDCs in Mississippi River and Lake Pontchartrain waters and sediments. The analytical protocol includes the following: procedures for cleaning glassware, labware, and glass fiber filters; procedures for isolating particulate and dissolved phase PPCP/EDCs; extraction procedures for particulate phase PPCP/EDCs; extraction procedures for PPCP/EDCs in the dissolved phase; extraction procedures for PPCP/EDCs in sediments; procedures for data reduction, validation and reporting; objectives and criteria for quality assurance/quality control; methods for assessing data precision, accuracy, representativeness, and completeness; and a listing of the physical and chemical characteristics of the eleven targeted PPCPs and EDCs.
- We identified eleven compounds as representative PPCPs and EDCs based on a telephone survey of local pharmacies in the New Orleans area. The selected

compounds are the following: a metabolite of a lipid regulator (clofibric acid); three analgesics (naproxen, ibuprofen and acetaminophen); two steroids (estrone and 17 β -estradiol); three fungicides and/or disinfectants (bisphenol-A, chlorophene and triclosan); an antidepressant (fluoxetine); and a human activity marker (caffeine). Analytical limits of detection ranged from <1 to 178 ng/L and are shown in the table below. Recoveries are based on surrogate compounds for each sample.

Target Compound	Type	Estimated Method DL (ng/L)*
Clofibric acid	Lipid regulator	3
Estrone	Steroid	3
17 β -Estradiol	Steroid	1
Ibuprofen	Analgesic	13
Naproxen	Analgesic	3
Acetaminophen	Analgesic	45
Bisphenol-A	Fungicide & disinfectant	0.6
Chlorophene	Fungicide & disinfectant	0.6
Triclosan	Fungicide & disinfectant	1
Fluoxetine	Antidepressant	178
Caffeine	Human activity marker	24

* Estimated method detection limit is based on instrument detection limits for a 2 μ L injection from a 100 μ L extract of a 1L sample and assuming 100 percent recovery.

- We developed and documented a detailed procedure for testing the analytical recovery of a water sample spiked with PPCPs and EDCs. The procedure includes the following: a list of laboratory supplies and chemicals; procedure for cleaning of glassware and labware; procedures for sample preparation; and procedures for sample analysis using GC/MS.
- Our sampling procedures and analytical methods can be used to develop environmental process-oriented research hypotheses related to the fate and transport of PPCPs and EDCs in the aqueous environment of southeastern Louisiana. In addition, our procedures and methods can be used to develop and test hypotheses related to the efficacy of treatment processes for the removal of PPCP and EDC contaminants from water resources and wastewater.

Publications, Manuscripts, Abstracts

Boyd, G.R. and D.A. Grimm. Occurrence of pharmaceutical contaminants and screening of treatment alternatives for southeastern Louisiana. Accepted for publication in *Environmental Hormones: The Scientific Basis of Endocrine Disruption, Annals of the New York Academy of Sciences* (expected 2001).

Boyd, G.R., S. Mitra and D.A. Grimm. GC/MS method for determination of PPCPs in aquatic samples. In preparation for submittal to *Chemosphere*.

Presentations

Grimm, D.A., S. Mitra and G.R. Boyd. 2001. Solid phase preparation for the analysis of pharmaceuticals in surface water and drinking water, *PITTCON 2001*, New Orleans, LA, March 5-8.

Boyd, G.R., H. Reemtsma and D.A. Grimm. 2001. Methods for determining the occurrence and effectiveness of treatment alternatives for PPCPs in raw and finished water supplies, *AWWA Annual Conference*, Washington DC, June 17-21.

Boyd, G.R. 2001. Pharmaceutical discharges into water. *Environment 2001: Water, Energy and the Law*, Tulane Environmental Law Society and the Tulane Institute for Environmental Law and Policy, New Orleans, LA, March 9-11. PowerPoint presentation is posted on a website hosted by the U.S. Environmental Protection Agency, Environmental Sciences Division, Completed Scientific Conferences Devoted to PPCPs in the Environment [<http://www.epa.gov/nerlesd1/chemistry/images/boyd1.pdf>].

Boyd, G.R. and D.A. Grimm. 2000. Occurrence of PPCP contaminants and screening of treatment alternatives for southeastern Louisiana, *Bioinformatics on the Bayou 2000*, Center for Bioenvironmental Research at Tulane and Xavier Universities, Tulane University Medical Center, New Orleans, Nov 16-17.

Boyd, G.R. 2000. Occurrence of pharmaceutical contaminants and screening of treatment alternatives for waters in southeastern Louisiana, *Environmental Hormone 2000*, Center for Bioenvironmental Research at Tulane and Xavier Universities, Tulane University Medical Center, New Orleans, Oct 16.

Intellectual Development

1. **Student Name(s):** Helge Reemtsma
2. **Funding Period:** October 2000 - May 2001
3. **Duties and Responsibilities:** Reemtsma prepared standard solutions for targeted PPCPs and EDCs and operated a gas chromatograph. Reemtsma also refined analytical procedures and analyzed samples to quantify PPCPs/EDCs in samples.

Useable Technologies

None

Repeat Acoustic Imaging of the Mississippi River Bed

Principal Investigator: Bernard J. Coakley, Ph.D.
Assistant Professor
Las Geology
Tulane University

Co-Investigator(s): Mead Allison, Ph.D.
Assistant Professor
Las Geology
Tulane University

Reporting Period: August 1999 - May 2001

Primary Objectives of Research Activities

To directly observe changes of elevation, sediment thickness and sediment texture in the Mississippi River using swath mapping and chirp sub-bottom profiling instruments, and relate these changes to the seasonal variations in river flow.

Progress Made to Achieve these Objectives

1.) We acquired the necessary mapping equipment and integrated it into a rational data acquisition system. Calibrated, upgraded and maintained these instruments as necessary.

After discussions with vendors and other scientists, three primary data acquisition systems were acquired. These are;

- Reson Seabat 8101 240 kHz swath mapping sonar
- TSS POS/MV ship's orientation and positioning system
- Edgetech SB 216S chirp towfish and X-Star data acquisition system

These data systems were supported by a data logging computer;

- Rack mount data logging computer with two flat panel screens

After we accumulated some experience with the system, we recognized certain deficiencies, which required additional equipment;

- 10/100 Base T Ethernet hub
- CSI MBX-3 DGPS Beacon Receiver
- Ashtech Z-FX dual frequency GPS receiver
- APC 1000W and 1400 W Uninterruptible Power Supplies
- CNS GPS time synchronizing clock
- Additional Flat Panel Screen for the Helm display

After the initial deployments on the R/V *Acadiana* from LUMCON, the equipment were permanently installed on the R/V *Eugenie*, Tulane's research ship.

2.) We conducted a series of cruises, mapping two segments of the Mississippi River. The study was expanded to include the Lower Atchafalaya River in 2001.

The upper section of the river, which extends from river mile 80 to 88 (approximately), was mapped in November, 1999, February 2000, May 2000, February 2001 and May 2001, spanning a weak flood season and a relatively strong flood (2001). The lower field area, extending from river mile 12 (near Venice, LA) to river mile 2 (near Pilot Town), was mapped in each campaign except the November 1999 cruise. These two areas are of contrasting form and appear to be formed by different processes. The lower area is a straight stretch of river which is, during low flow, primarily estuarine. The upper area is the last broad curve of the river as it approaches the gulf. Processes here are more typical of meandering streams, the bottom is typically sandy.

In the February and May 2001 cruises, mapping surveys were also conducted along a seven-mile section of the lower Atchafalaya River to compare and contrast this major Mississippi distributary with the main river. This section corresponds in distance upstream and bottom conditions with the estuarine, lower Mississippi section (mile 12 to 2).

3.) We collected bottom samples for sonar ground truth and conducted analyses of sediment texture, composition and isotope geochemistry.

Grab samples were collected during each cruise for the upper and lower field areas. These samples, collected with a Kalisco Smith-McIntyre grab sampler, were obtained to examine the true surface sediment composition and texture (e.g., grain size) in order to ground truth seismic data. All grab samples were analyzed for grain size using sieve and pipette analysis. These data were directly compared to side-scan sonar mosaics to determine what effect changes in bottom texture have on acoustic return strength. Several 3-m-long kasten corers were also collected from the lower field area where thick accumulations of fine-grained sediments were found on the river bed during the low discharge periods. These samples were x-rayed to examine internal stratigraphy and measured for short-period radioisotopes (Be-7, Pb-201, Cs-137) to examine the sedimentation history of the layers. This stratigraphic data were compared to CHIRP subbottom seismic profiles of the same areas.

Major Accomplishments

- Repeat mapping of two segments of the river during a low flow (2000) and a more typical flow year (2001).
- Comparative mapping of the lower Mississippi and lower Atchafalaya distributaries.
- Direct observation of cyclic sediment storage of fine-grained sediment in the lower Mississippi River and Atchafalaya River mapping areas.
- Direct observation of sand wave migration in the upper river mapping area.

- Initiation of a study (at no charge to this grant) of changes in the river bed over the last century, using periodic hydrographic surveys collected by the Army Corps of Engineers.

Publications, Manuscripts, Abstracts

Allison, M.A., and B.J. Coakley, 2001, Seasonal And Interannual Storage Of Fine-Grained Sediment In The Lower Mississippi River: Evidence From Acoustic Mapping And Bottom Sampling, American Society of Limnology and Oceanography Abstracts, p 17.

Campanella, R*, B.J. Coakley, and M.A. Allison, 2001, Historical Bathymetry From The Army Corps Of Engineers For The Lower Mississippi River, 1915-1992: Evolution Of A Controlled River, American Society of Limnology and Oceanography Abstracts, p 30.

Coakley, B.J., and M.A. Allison, 2001, Repeat Swath Mapping At English Turn; Direct Imaging Of Bedform Migration And Sediment Storage In The Lower Mississippi River, American Society of Limnology and Oceanography Abstracts, p 36.

Presentations

The above abstracts were presented at the ASLO meeting in Albuquerque, New Mexico in February 2001. Abstracts can be found on line at;
<http://www.aslo.org/albuquerque2001>.

Intellectual Development

1. **Student Name(s):** John Galler
2. **Funding Period:** John Galler is funded by a departmental PhD fellowship.
3. **Duties and Responsibilities:** John Galler participated in the February-March 2001 and May 2001 cruises on the R/V *Eugenie*. He is interpreting the data from the Venice mapping area and compiling maps of his interpretations as part of his dissertation research.

Useable Technologies:

None.

*Richard Campanella is the GIS support technician for the Center for Bio-Environmental Research at Tulane. He has put the various historical hydrographic surveys for the Army Corps of Engineers in a common spatial reference and performed a variety of data quality checks. Working with Coakley and Allison, he will analyze the data for changes in the Mississippi River bed over the last century.

Generic Model to Embed Intelligence in Environmental Sensors

Principal Investigator: Fernando Figueroa, Ph.D.
Assistant Professor
Mechanical Engineering Department
Tulane University

Reporting Period: August 1999 - December 2000

Primary Objectives of Research Project

This grant has made possible research to develop a generic methodology to embed "intelligence" into sensor models. The objective was to instantiate bio-sensors as highly-autonomous entities able to monitor themselves and the processes in which they partake in order to ensure acquisition of high integrity data and to monitor and assess the health of the sensor processes. A particular incentive was to develop applications for biosensors to be deployed in autonomous underwater vehicles, or installed in remote and/or areas of difficult accessibility.

A method was developed to turn any sensor into an intelligent entity capable of interpreting data as a sequence of qualitative behaviors (trends based on qualitative models derived using first principles). This approach was inspired by the techniques employed by operators who are tasked to monitor signals of sensors, and to use that information to reason and make decisions.

Furthermore, sensor fusion strategies were developed to take advantage of the rich qualitative information provided by the intelligent sensor models. The fusion techniques were based on employing qualitative reasoning around a process in which multiple intelligent sensors participate.

Detailed information on the accomplishments of the research supported by this grant is available as publications (listed below).

Publications, Manuscripts and Abstracts

Articles submitted to Refereed Journal Publications

Figueroa, Fernando and Yuan, Xiaojing, "Highly Autonomous Sensors and Qualitative Physics: Modeling and Implementation", *IEEE/ASME Transactions on Mechatronics*, November 2000.

Papers in Refereed Conference Proceedings

Figueroa, F. and Yuan, X., "Sensor Fusion for a Network of Processes/Systems with Highly Autonomous Sensors," *IEEE International Workshop on Virtual and Intelligent Measurement Systems*, Budapest, Hungary, May 19-20, 2001, pp 4-10

Figueroa, F. and Yuan, X., "A Sensor Fusion Method for a Network of Highly Autonomous Sensors," *International Mechanical Engineering Congress and Exposition*, November 5-10, 2000, Orlando, Florida

Figuroa, F. and Yuan, X., "A Taxonomy and Environment to model any sensor as highly autonomous sensor," International Workshop on Virtual and Intelligent Measurement Systems, April 29-30, 2000, Annapolis, MD, pp 75-82

Articles submitted to Refereed Conference Publications

Yuan, J.X., and Figuroa, F., "Intuitive Intelligent Sensor Fusion with Highly Autonomous Sensors," 2001 IMECE, Symposium on Intelligent Sensors, November 11-16, 2001, New York, NY
(Accepted)

Intellectual Development

1. **Student(s) Name:** Xiaojing Xyuan, Ph.D. and Yuebo Ma
2. **Period of Funding:** December, 2001 and August, 2001 respectively
3. **Duties and Responsibilites:** Both students worked on the following theses and projects

Xiaojing, "Fusion Theory for a Network of Highly Autonomous Sensors"

Yuebo, "Wavelet Transform Applications to Qualitative Interpretation of Sensor Data"

Useable Environmental Technologies

None.

Developing Chemosensors Based on Self-Assembling Induced Fluorescence Enhancement

Principal Investigator: Chao-Jun Li, Ph.D.
Professor
Department of Chemistry
Tulane University

Co-Investigators: Russell H. Schmehl, Ph.D.
Professor
Department of Chemistry
Tulane University

Reporting Period: May 1999 – April 2001

Summary of Progress:

Previously, we have studied highly selective sensors for potassium, sodium, and cesium. During the period of this initiation project, we have designed and studied the sensing of lead, cadmium and mercury ions with fluorescence-based chemosensors. A focus of this period is the sensing of lead ions in aqueous media. We found that a sensor previously used for potassium in organic solvent under anhydrous conditions has a high and exclusive selectivity toward lead ions in aqueous conditions. Detailed studies were carried out to determine the mechanism of the leading sensing in aqueous conditions. It was shown that depending on the counter-ions associated with lead, either two by two sandwich or one by one sandwich structures are possible and are able to cause fluorescence enhancement. In addition, crystal structures of these complexes were grown and the structures were determined by X-ray diffraction. The study confirms our previous proposed mechanism for the new class of fluorescence sensors. In addition, efforts were also made to coat the sensor molecules to glass surface in order to make device.

Primary Objectives of Research Project

The primary objectives of the research project are: (1) to study the sensing mechanism of self-assembling induced enhancement, (2) to develop chemosensors to analyze heavy metal ions, (3) to investigate techniques to ensemble the sensor molecules on glass surface to make devices.

Progress Made to Achieve these Objectives

During this period, we have been able to decipher the sensing mechanism via various instrumental and physical experiments. The studies clearly proved that self-assembling is the determining factor that governs the sensing properties of the sensors

During this period, new sensors were developed to selectively detecting heavy metal ions including mercury, lead, cadmium and copper.

Various techniques were explored to attach the sensor molecules onto glass surfaces.

Major Accomplishments

- obtained x-ray crystal structures of the sensor molecules and lead ions,
- determined the life-time of the excited states of the sensor molecules in the free form and in the bonded form
- developed sensors for lead, mercury, cadmium, and copper,
- made preliminary device-making studies related attaching the sensor molecules onto glass surface

Publications, Manuscripts and Abstracts

Xia, W. S.; Schmehl, R. H.; Li, C. J. "A Fluorescent 18-Crown-6 Based Luminescence Sensor for Lanthanide Ions" *Tetrahedron* 2000, 56, 7045.

Xia, W. S.; Schmehl, R.; Li, C. J. "A Novel Fluorescent Chemosensor for Cesium Ions" *Chem. Commun.* 2000, 695. (News Roundup in *CHEMWEB*, by Bradley, D. April 10, 2000; Selected as the most popular research article of August 2000 by the Royal Society of Chemistry).

Xia, W. S.; Schmehl, R.; Li, C. J. "Synthesis and Study of a Novel Potassium Sensor", *Eur. J. Org. Chem.* 2000, 387.

Xia, W. S.; Schmehl, R. H.; Li, C. J.; Mague, J. T.; Luo, C. P.; Guldi, D. M. "Chemosensors for Lead (II) and Alkali Ions Based on Self-Assembling Fluorescence Enhancement (SAFE)" *J. Phys. Chem.* 2001, 0000.

Presentations

Xia W. S.; Schmehl, R. H.; Li, C. J. "Chemosensors based on SAFE", ACS National Meetings, Washington DC, August 2000.

Li, C. J. "Photons, Electrons, and Water", invited lecture, Louisiana State University, 2000.

Li, C. J. "Photons, Electrons, and Water", invited lecture, University of South Alabama, 2000.

Li, C. J. "Photons, Electrons, and Water", invited lecture, Colorado State University, 2000.

Intellectual Development

1. **Student Name:** Xian-Yong Wang, Graduate student
2. **Funding Period:** Jan. 2000-April 2001:
3. **Duties and Responsibilities:** Synthesizing sensor molecules and studying the attachment of sensors to surface

1. **Student Name:** Chris Costellio, Graduate student
2. **Funding Period:** May 1999-April 2001 (part-time)
3. **Duties:** Attaching sensors to surface.

1. **Student Name:** Wen-Chun Zhang, Post-Doc
2. **Funding Period:** May 1999-Dec. 1999 (Part-time)
3. **Duties:** Synthesizing sensor molecules

1. **Student Name:** Wen-Sheng Xia, Post-Doc
2. **Funding Period:** May 1999-Dec. 2000
3. **Duties:** Synthesizing sensor molecules, studying sensing, developing devices

1. **Student Name:** Taisheng Huang, Post-Doc
2. **Funding Period:** Sept. 1999-April 2001 (part-time)
3. **Duties and Responsibilities:** Synthesizing sensor molecules

1. **Student Name:** J. Laflour, Undergraduate student
2. **Funding Period:** Sept. 2000-Dec. 2000 (part-time)
3. **Duties and Responsibilities:** Attaching sensors to surfaces

Useable Environmental Technologies

Potential new sensor devices for ca, K, Na, Hg, Cd, Pb, Cu for environmental and bio-medical applications.

River-Ocean Interactions (Phase I): The Processing and Fates of Nutrients and Organic Carbon from the Mississippi River

Principal Investigator: Brent McKee, Ph.D.
Professor
Geology Department
Tulane University

Co-Investigators: Tom Bianchi, Ph.D.
Associate Professor
EE Biology
Tulane University

Mike Dagg, Ph.D., Professor
Louisiana Universities Marine Consortium

Richard Miller, Ph.D., Chief Scientist
NASA Stennis Space Center

Rodney Powell, Ph.D. Assistant Professor
Louisiana Universities Marine Consortium

Reporting Period: July 1999 – April 2001

Primary Objectives of Research Project

To use a combination of remote sensing, biological, and geochemical techniques to determine the major pathways for terrestrial carbon from the Mississippi River and marine carbon produced from nutrients delivered by the Mississippi River.

Progress Made to Achieve these Objectives

Great progress was achieved for this “proof-of-concept” project. The major pathways for terrestrial carbon from the Mississippi River have been determined for two seasonal time frames in 2000 (Spring-high flow; Fall low flow). These two field experiments have resulted in a large volume of data that was collected by all five investigators under strict conditions of coordination so that a major portion of the carbon pathway can be understood. A number of high quality manuscripts have been (and will continue to be) produced and published in peer-reviewed journals.

Major Accomplishments

- First major field effort was successfully completed in-April 2000; samples were collected during this 10-day research cruise to characterize the Spring (high discharge) season in the study area.
- Web page (www.lumcon.edu/mirir) was established to report our major finding to the public, share our data with other researchers and to enhance data sharing between PIs.
- First Data workshop held at Tulane in September 2000 to begin the process of achieving a coordinated understanding of carbon pathways in the study area.
- Second major field effort was successfully completed in October 2000; samples were collected during this 10-day research cruise to characterize the Fall (low discharge) season in the study area.

- McKee and Bianchi co-chaired an all-day session at a major national meeting (ASLO; American Society of Limnology and Oceanography) focused on river-ocean processes. McKee presents a talk that outlines the initial overall synthesis of the project. Five other posters were presented detailing findings of the project.

Publications, Manuscripts and Abstracts

- Chen, N., T. Bianchi, B. McKee, and J. Bland. Historical Trends of Hypoxia on the Louisiana Shelf: the Application of Pigments as Biomarkers. *Organic Geochemistry*. 32 (4): 543-561 2001
- Miller, R. Measuring CDOM using a Multiple Pathlength Liquid Waveguide System. *Continental Shelf Research*. Submitted July 2001
- Chen, N., T. Bianchi, and J. Bland. Novel sediment pigment biomarkers as indicators of grazing on the Louisiana shelf: The application of high-performance liquid chromatography-mass spectrometry (HPLC-MS) techniques. *Marine Chemistry*. In Preparation.
- McKee, B., R. Corbett and D. Duncan. Sediment deposition and redistribution in the Mississippi River Bight. *Continental Shelf Research*. In Preparation
- Corbett, R., B. McKee and D. Duncan. The fate of particle-reactive radionuclides on a river-dominated margin: The Mississippi River and adjacent shelf. *Geochimica et Cosmochimica Acta*. In Preparation
- McKee, B., T. Bianchi and R. Corbett. The deposition and preservation of terrestrial and marine organic carbon in the ocean margin adjacent to the Mississippi River. *Geochimica et Cosmochimica Acta*. In Preparation
- Wysocki, L., T. Bianchi, and B. McKee. Sources and spatial variability of terrestrial organic matter in sediments within the depositional flow path of the Mississippi River Plume. *Geochimica et Cosmochimica Acta*. In Preparation
- Wysocki, L., T. Bianchi, R. Miller, and R. Powell. Spatial dynamics of particulate and dissolved organic carbon in the Mississippi River Plume: Effects of flow regime. *Limnology and Oceanography*. In Preparation
- Wysocki, L., and T. Bianchi. Spatial variability in the composition and relative abundance of amino acids in the total nitrogen pool within the Mississippi River Plume. *Limnology and Oceanography*. In Preparation
- Powell, R. Distribution of Fe complexing ligands in the Mississippi River Plume. *Estuarine and Coastal Shelf Science*. In Preparation
- Powell, R. and A. Finelli-Wilson. Nutrient distributions in the Mississippi River Plume. *Estuaries*. In Preparation
- Miller, R. C. Hall, C. Del Castillo, and B. McKee. Bio-optical properties associated with the Mississippi River plume. *Journal of Geophysical Research*. In Preparation
- Hall, C., R. Miller and B. McKee. Relationship between the Fluorescence Lifetime of Chlorophyll a and Primary Productivity within the Mississippi River Plume. *Journal of Plankton Research*. In Preparation
- Miller, R., C. Hall and S. Fernandez. Estimates of Phytoplankton Photochemical Efficiency Derived from Fluorescence Lifetime Measurements *Journal of Plankton Research*. In Preparation
- Chen, N., T. Bianchi, B. McKee, and J. Bland. Fate of chlorophyll-a in the lower Mississippi River and Louisiana shelf: Effects of differential sedimentation and redox. *Geochimica Cosmochimica Acta*. In Preparation

- Bianchi, T. Mitra, S., and B. McKee. Sources of terrestrially-derived organic carbon in the lower Mississippi River and inner Louisiana shelf: Implications for differential sedimentation and transport. *Marine Chemistry* In Preparation
- Dagg, M.J. and H. Liu. Grazing by the microzooplankton and mesozooplankton communities in the vicinity of the Mississippi River plume during spring and fall 2000. *Marine Ecology Progress Series*. In Preparation
- Urban-Rich, J. and M.J. Dagg. The potential contribution of mesozooplankton fecal pellet production to carbon flux in the vicinity of the Mississippi River plume during spring and fall 2000. *Marine Ecology Progress Series*. In Preparation
- Liu, H. and M.J. Dagg. Bacterioplankton and picoplankton distribution and abundance in the vicinity of the Mississippi River plume during spring and fall 2000. *Journal of Plankton Research*. In Preparation

Presentations

- Carbon Cycling and Burial in the Mississippi Delta Region. B. McKee, M. Dagg, T. Bianchi, R. Corbett, R. Powell. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.
- Fate of Chlorophyll-A in the Lower Mississippi River and Louisiana Shelf: Effects of redox. N. Chen, T. Bianchi and B. McKee. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.
- Effect of Seasonal Sediment Storage and Diagenesis in the Lower Mississippi River bed on Bioavailability of Particulate Phosphorus Flux to the Gulf of Mexico. M. Satula, T. Bianchi and B. McKee. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.
- Bio-optical Properties of the Mississippi River Plume and Adjacent Shelf, R. Miller, C. Hall, C. Del Castillo, J. Yuan, B. McKee and M. Dagg. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.
- Nutrient Cycling in the Mississippi River Plume. A. Wilson-Finelli and R. Powell. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.
- Microzooplankton Grazing of the Phytoplankton in the Mississippi Plume. M. Dagg and H. Liu. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.
- Organic Complexation and Speciation of Iron in the Mississippi River Plume. R. Powell and A. Wilson-Finelli. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.
- Naturally-Occurring Radioisotopes as Indicators of Sediment Cycling in River-Dominated Ocean Margins. R. Corbett, B. McKee and D. Duncan. 2001 Aquatic Sciences Meeting sponsored by the American Society of Limnology and Oceanography; February 2001, Albuquerque NM.

Intellectual Development

1. **Student Name:** Laura Wysocki
2. **Period of funding** 1 July 1999 – 30 April 2001
3. **Brief description of duties and responsibilities:** collect samples during both field experiments, perform analyses on organic constituents

Useable Technologies

None

Autonomous Monitoring and Visualization Technology Development for Aquatic Environments

Principal Investigator: Douglas J. Meffert, Ph.D.
Clinical Associate Professor
Associate Director for Planning
Center for Bioenvironmental Research

Funding Period: September 1999 - April 2001

Summary of Progress

Primary Objectives of Research Project

The increased prevalence and threat of chemical warfare agents have necessitated advanced warning of the presence of these harmful constituents in the air, water, and soil. The Tulane/Xavier Center for Bioenvironmental Research has been developing for the past several years biosensors to detect the compounds of interest to the Navy. The CBR has employed a variety of innovative biologically based receptors, which utilize biologic reactions to assess and report the types and quantity of toxins in the field. These projects employ a variety of methods including field studies and in vitro and in vivo laboratory studies at Tulane and Xavier Universities with some of the projects already employing initial field demonstrations.

The significance of this research includes development of new technologies, which will allow for cost-effective assessments of toxicity in aquatic environments of interest to the DOD/DTRA. A remaining need for this program is the integration and co-development of technologies to deploy these biosensors in remote environments. The safest and most cost-effective means in aquatic environments would be through autonomous/unmanned underwater vehicles (AUV's). **Our goal is to become the first entity in the nation to produce a biosensor for effective deployment on an AUV (tentatively scheduled for Fall 2001).**

Progress Made to Achieve These Objectives

We have made significant progress in realizing our goal of having the first biosensor deployed on an AUV. Mr. Bernard has conducted an assistantship at NAVOCEANO and provides linkage and assistance in research development (i.e., modeling and field testing) for the CBR and NAVOCEANO during the past several years. Through this relationship, we have determined our AUV of choice, the REMUS vehicle being developed by Woods Hole Oceanographic Institute, as a platform for one of our antibody-based biosensors (being developed by Drs. Diane Blake and Robert Blake of Tulane and Xavier, respectively).

For this project, I sought support to hire a computer graphics technician in the CBR's visualization laboratory to provide graphic support for computer/Internet visualization of aquatic monitoring results made possible through AUV modeling and field testing through this project.

I also sought support for John Bernard as a graduate research assistant at Tulane's School of Engineering, Mechanical Engineering Department to help conduct research on AUV's.

Major Accomplishments

- Facilitated interactions to promote coordinated research between Tulane and Xavier investigators; Naval Oceanographic Office, Stennis, MS; and private sector (COTS Technology, LLC and Sapidyne Instruments) for joint research on biosensor coupling with autonomous underwater vehicle navigation enhancement and "intelligent" programming for long-term deployment, accuracy, and self-regulation of biosensors in aquatic environments.

Publications, Manuscripts and Abstracts

Warrenfeltz, L., McLachlan, J., Meffert, D., and Rey, G: A LEAG of Their Own: A Partnership for the Mississippi River-Gulf of Mexico System. Proceedings of the MTS/IEEE Oceans 2000 Conference, Providence, RI, September 2000.

Meffert, D.J., D.A. Blake, R.C. Blake II, and R.G. Rey: Biosensor Development for Autonomous Real-Time Monitoring of Environmental Toxicants, Proceedings of Autonomous Undersea Systems Institute: Sensors and Sensing Technology for Autonomous Ocean Systems Workshop, Waikoloa, HI, October 2000.

Presentations

Meffert, D.J.: Biosensor Development for Autonomous Real-Time Monitoring of Environmental Toxicants. Invited presentation at the Sensors and Sensing Technology for Autonomous Ocean Systems Workshop. Sponsored by the Office of Naval Research and the Autonomous Undersea Systems Institute, Waikoloa, HI, October 29, 2000.

Invited by Autonomous Undersea Systems Institute to present paper at the 12th Annual Unmanned, Untethered Submersible Technology Workshop in New Hampshire, August 2001.

Intellectual Development

1. **Student(s) name:** John Bernard, Mechanical Engineering Department, School of Engineering, Tulane University, New Orleans, LA.
2. **Period of funding:** 1/1/99-12/31/00
3. **Brief description of duties and responsibilities:** help conduct research on AUV's

Useable Environmental Technologies:

1. **Title of technology product**
Integrated autonomous immunosensor and autonomous underwater vehicle system.
2. **Description of technology product**
Facilitated interactions to promote coordinated research between Tulane and Xavier investigators; Naval Oceanographic Office, Stennis, MS; and private sector (COTS Technology,

LLC and Sapidyne Instruments) for joint research on biosensor coupling with autonomous underwater vehicle navigation enhancement and "intelligent" programming for long-term deployment, accuracy, and self-regulation of biosensors in aquatic environments.

3. Utility/benefit/ROI/payoff of technology product

Enhanced real-time biosensor deployment for environmental compliance and, ultimately biologic warfare detection.

4. Timeline (demonstration, validation, completion, etc.)

AUV navigation study to be completed in January 2001. Biosensor to be deployed on AUV or stationary buoys in fall 2001.

5. Partners (academia, industry, labs/centers, federal agency, etc.)

Naval Oceanographic Office

Tulane and Xavier Universities

Sapidyne Instruments, Boise, Idaho

COTS Technology, LLC, New Orleans, LA

Woods Hole Oceanographic Institute, Woods Hole, Massachusetts

6. Patents (applied for and issued)

Patents will be applied for by partners

Sedimentation and Resuspension Studies for the Mississippi River and Louisiana Coastal Environments

Principal Investigators: Efstathios E. Michaelides, Ph.D.
Associate Dean - Engineering
Tulane University

Co-Investigator(s): Laura J. Steinberg, Ph.D.
Assistant Professor
Civil and Environmental Engineering
Tulane University

Elia Eschenazi, Ph.D.
Chair and Professor,
Department of Physics
Xavier University

Reporting Period: August 1999 - June 2001

Primary Objectives of Research Activities

To investigate the sedimentation and resuspension characteristics of single particles and flocs in aqueous solutions.

Progress Made to Achieve these Objectives

1. To investigate the sedimentation characteristics

We have performed computational and experimental studies that yield information on the sedimentation behavior of single particles as well as clusters of particles. In the computational area we have developed a code, which utilizes the Lattice-Boltzman Method (LBM). The method is the most recently developed technique in Computational Fluid Dynamics and enables us to consider the dynamics of single particles as well of groups of particles. So far our code has been used to simulate the sedimentation process of up to 200 particles and we are extending its capabilities to 1000 particles. In the experimental area we have constructed a facility to measure the drag coefficients of spheres as well as irregular particles in viscous fluids. The range of the Reynolds numbers of the experiments is from 0.001 to 2000.

2. To investigate the resuspension characteristics

We have performed computational studies that yield information on the sedimentation behavior of single particles as well as clusters of particles. In the computational area we have developed a code, which utilizes the Lattice-Boltzman Method (LBM). The method is the most recently developed technique in Computational Fluid Dynamics and enables us to consider the dynamics of single particles as well of groups of particles. Our code has been used to determine the lift force exerted by the flow on single particles present at the bottom of rivers and lakes as well as in determining the resuspension patterns and behavior of groups of particles.

Major Accomplishments

- We have completed a code based on the LBM to study the sedimentation and resuspension behavior of single particles and groups of particles.
- We have determined the lift/resuspension force of a particle sitting in the bottom of a flow, which carries a suspension of similar particles.
- We have determined the sedimentation velocities and drag coefficients of particles settling in a chamber.
- We have determined the lift force on a moving particle that is close to a wall.
- We have found out that the hydrodynamic lift forces exerted by a suspension of particles on a stationary particle are sufficient to cause the resuspension of the latter without any inter-particle collisions.
- We have found out that sedimentary particles strongly interact and form dynamic clusters. The characteristics of the sedimentation of the clusters are totally different than those of single particles.

Publications, Manuscripts, Abstracts

Feng, Z.-G. and Michaelides, E. E., "Inter-particle forces and lift on a particle attached to a solid boundary in suspension flow," Physics of Fluids, accepted for publication, 2001.

Feng, Z.-G. and Michaelides, E. E., "Hydrodynamic Force on Spheres in Cylindrical and Prismatic Enclosures," Int. J. Multiphase Flow, accepted for publication, 2001.

Tsega, Y., Michaelides, E. E. and Eschenazi, E. V., "Particle dynamics and mixing in the frequency driven 'Kelvin cat eyes' flow," Chaos, in print, 2001.

Tran-Cong, S., Feng, Z.-G. and Michaelides, E. E., "A new model for the transport of suspended sediment," J. Hydraulic Eng., accepted for publication, 2001.

Feng, Z.-G. and Michaelides, E. E., "Drag coefficients of viscous spheres at intermediate and high Reynolds numbers," J. Fluids Eng., in print, 2001.

Presentations

Feng, Z.-G. and Michaelides, E. E. "Viscous droplets in gaseous streams," Symposium on the 100th anniversary of the NY Polytechnic Institute, New York, NY, November 1999.

J. R. Martin, Jr., L. J. Steinberg, and E. E. Michaelides "Determination of Bed Shear Stress by Digital Particle Image Velocimetry in Turbulent Open Channel Flow," Joint Conference in Water Resources Engineering and Water Resources Planning & Management, Minneapolis, MN August 2000.

Z.-G. Feng, L. J. Steinberg, E. E. Michaelides, "Transport of dissolved contaminants within a stream bed with bedforms" Joint Conference in Water Resources Engineering and Water Resources Planning & Management, Minneapolis, MN August 2000.

Feng, Z.-G. and Michaelides, E. E., "Drag coefficients of viscous drops," Proc. Of the ASME-FED-2000, pp. 709-719, November 2000.

Tran-Cong, S., Feng, Z.-G. and Michaelides, E. E. "A novel method for sediment transport based on the equations of turbulence," APS-DFD annual meeting, Washington DC, November 2000.

Feng, Z.-G. and Michaelides, E. E., "Fluid dynamics of a sphere in an arbitrary electric field," 4th International Conference on Multiphase Flow, New Orleans, LA, May 2001.

Tsega, Y., Michaelides, E. E. and Eschenazi, E., "Particle dynamics and mixing in the frequency driven 'Kelvin cat eyes' flow," 4th International Conference on Multiphase Flow, New Orleans, LA, May 2001.

Intellectual Development

1. **Student Names:** Zhi-Gang Feng, post-doctoral student
Sabine Tran-Cong, post-doctoral student
Xujia Xu, Ph.D. Student, Mechanical Engineering
Jim Martin, Ph. D. Student, Civil and Environmental Engineering
Swirvine Niyendra, Ph. D. Student, Civil and Environmental Engineering
Lorenzo Craig, BS student, Physics-Xavier
Michelle Wyche, BS student, Physics-Xavier
2. **Funding Period:** August 8, 1999 to June 30, 2001
3. **Duties and Responsibilities:** Drs. Michaelides, Steinberg and Eschenazi were in charge of the direction of the research team and the advisement of the graduate and undergraduate students. Dr. Feng assisted with the computational aspects and helped in the advisement of Messers Xu, Craig and Wyche. Dr. Tran-Cong assisted with the experimental part of the research and helped with the advisement of Messers Martin and Niyendra.

Useable Technologies

The LBM computer code that can be used for the simulation of particle flow.

Emission Monitoring/Analysis of Diesel Fuels in a Shock Tube

Principal Investigator: J. Yao, Ph.D.
Professor
Department of Chemistry
Xavier University of Louisiana

Reporting Period: August 1999 – September 2000

Summary of Progress:

The commercial fuel contains 60% parafins, 25% aromatics, and 15% olefins. The following table lists the different aspects of properties of these components.

	Ignition quality	Heat value	Smoking tendency
Parafins	Good	Low	Low
Aromatics	Poor	High	High
Olefins	fair	low	moderate

As we can see from the above table, the aromatics are the major concern with respect to particulate carbon formation but they have a higher heat value which provides more power for the engine. The ignition quality is measured by the cetane number. The higher the cetane number, the better the ignition quality. N- hexadecane ($C_{16}H_{34}$) has a cetane number of 100. α -methyl naphthalene ($C_{10}H_7CH_3$) has a cetane number of 0. The parafins, aromatics and olefins can be mixed at various compositions to produce diesel fuels of different properties.

The present trend of the usage of the unleaded gasoline has tended to increase the aromatics content of the fuel. The waste gas of diesel engines contains a considerable portion of polyaromatic hydrocarbons (PAH) and particulate organic carbons (POC). This widespread increase in the aromatic content of the unleaded fuels has created a potential problem to the human health as well as to the environment. During combustion, extensive pyrolysis of hydrocarbons takes place leading to in chamber soot formation (wall deposits) which may lead to smoke emission. There is now considerable experimental evidence to show that soot formation in practical flames proceeds mainly through PAH formation; this explains the difference between the sooting tendency of aromatic and non aromatic fuels, the former giving greater amounts of PAH and therefore more soot than the latter.

Primary Objectives of Research Project

We would be theoretically (through earlier studies on many aromatic compounds) trying to find how the PAH and POC can be suppressed and also understand the chemical transformations and the products they produce. We can thereby identify why engines knock and how to minimize pollution.

Kinetic Modelling

An understanding of all the chemical processes taking place during soot formation can be achieved only by kinetic modelling. However, the accurate prediction of soot formation is still a formidable problem,

because of the uncertainties about the factors governing the pyrolysis and oxidation reactions, in particular those concerning the aromatic compounds, which are very important in soot formation, as well as lack of reliable thermochemical data. Those species which do not have thermochemical data will be established with their spectroscopic properties using a special computer program. Then these reactions and their thermochemical data will be tested using CHEMKIN and a kinetic model is proposed in this study.

Progress Made to Achieve these Objectives

Studies on the pyrolysis of few organic compounds like Pyrazine, n-heptane, toluene-n-heptane, toluene-iso-octane have been studied in order to find a theoretical reaction process for the pyrolysis of N-hexadecane.

In order to develop an experimental model for the above mentioned sample a large number of free radical reactions were approximated by a molecular model consisting of a primary and secondary reactions.

REACTIONS	LogA ⁰	n	E _a
C4H4N2 + M = C4H3N2 + H + M	1.83E+7	0.00	106100
C4H3N2 = C2H2 + NCCHN	1.63E+5	0.00	30000
NCCHN + M = H + C2N2 + M	2.47E+16	-6.45	44120
NCCHN + M = HCN + CN + M	3.00E+18	-7.73	50260
CN + C4H4N2 = 2HCN + CHCHCN	1.62E+6	0.00	0.00
CHCHCN + M = CHCCN + H + M	1.42E+20	-8.55	64040
CHCHCN + M = C2H2 + CN + M	6.29E+25	-11.91	84190
H + C4H4N2 = C4H3N2 + H2	2.68E+5	0.00	900
CN + C4H4N2 = C4H3N2 + HCN	365	2.30	0.00
CHCHCN + C4H4N2 = CH2CHCN + C4H3N2	55	2.40	0.00
CN + HCN = H + C2N2	2695	1.57	0.00
CN + H2 = HCN + H	15.5	3.18	-220
CN + C2H2 = CHCCN + H	8.62E+9	-3.00	0.00
C2H + HCN = H + CHCCN	8.62E+9	-3.00	0.00
C2H + HCN = CN + C2H2	2.62E+10	-3.00	0.00
C2H + C2H2 = C4H2 + H	1.30E+6	0.00	0.00
CH2CHCN + H = CHCHCN + H2	336.8	2.53	6000
CH2CHCN + CN = CHCHCN + HCN	364.8	2.30	0.00
H2 + C2H = C2H2 + H	273	2.40	200
C2N2 + C2H = CHCCN + CN	8.05E+5	0.00	0.00
H + NCCHN = H2 + C2N2	1.09E+6	0.00	0.00
H + NCCHN = 2HCN	9.83E+5	0.00	0.00
CN + NCCHN = HCN + C2N2	1.62E+6	0.00	0.00
H + CHCHCN = H2 + CHCCN	1.09E+6	0.00	0.00
H + CHCHCN = HCN + C2H2	9.83E+5	0.00	0.00
H + CHCHCN = CH2CHCN	1.63E+5	0.00	0.00
CN + CHCHCN = HCN + CHCCN	1.62E+6	0.00	0.00
CN + CHCHCN = C2N2 + C2H2	9.83E+5	0.00	0.00
2CHCHCN = CHCCN + CH2CHCN	8.05E+5	0.00	0.00
NCCHN + CHCHCN = C2N2 + CH2CHCN	8.05E+5	0.00	0.00
C2H + CN + M = CHCCN + M	3.26E+7	0.00	0.00
C2N2 + M = 2CN + M	2.09E+7	0.00	100310
HCN + M = H + CN + M	6.31E+6	0.00	105210
CH2CHCN = C2H2 + HCN			

An understanding of all these chemical processes taking place during soot formation can be established only by kinetic modeling therefore these reactions were tested using the chemkin software for predicting the chemical behavior behind incident and reflected shock waves. However due to lack of reliable thermochemical data for some of the intermediate reaction species (those underlined) we could not test them successfully. However, efforts are being made to establish the thermochemical data by using the spectroscopic properties of the species through their C_p/R , H^0/RT , S^0/R values.

Major Accomplishments

None

Publications

None

Presentations

None

Intellectual Development

None

Useable Environmental Technologies

None

APPENDIX B.

PUBLICATIONS, ABSTRACTS & PRESENTATIONS

Publications, Abstracts and Presentations

Publications

Basavapathruni, Radha - Masters Degree Student, Dept. Environmental Health Science, Tulane University. Molecular Docking and Molecular Dynamics Simulations of Endocrine Disrupting Chemicals. In preparation for submission as partial fulfillment of the requirements for a Master of Public Health.

Bianchi, T. Mitra, S., and **B. McKee**. Sources of terrestrially derived organic carbon in the lower Mississippi River and inner Louisiana shelf: Implications for differential sedimentation and transport. *Marine Chemistry*. In Preparation

Bishop, Thomas C and Zhmudsky, Oleksandr O. Mechanical Model of Nucleosome and Chromatin Dynamics (submitted)

Bishop, Thomas C and Zhmudsky, Oleksandr O. Elastic Wave Propagation Along DNA. Los Alamos National Labs E-Print Archive. arXiv:physics/010171 v3 .

Bishop, Thomas C and Zhmudsky, Oleksandr O. Information Transmission along DNA. *Currents In Computational Molecular Biology 2001*. Pg 105-106. Les Publications CRM, Montreal. Eds. N. El-Mabrouk, T. Lengauer, and D. Sankoff.

Bishop, Thomas C., Williams, Kirk Y., Hall, Andrew. Dynamics of Estradiol and DES in solution and bound to the estrogen receptor. (in preparation)

Blake, D.A., R.M. Jones, R.C. Blake II, A.R. Pavlov, I.A. Darwish, and H. Yu (2001) "Antibody-based sensors for heavy metal ions", *Biosens. Bioelectron.*, in press.

Boyd, G.R. and D.A. Grimm. Occurrence of pharmaceutical contaminants and screening of treatment alternatives for southeastern Louisiana. Accepted for publication in *Environmental Hormones: The Scientific Basis of Endocrine Disruption, Annals of the New York Academy of Sciences* (expected 2001).

Boyd, G.R., S. Mitra and D.A. Grimm. GC/MS method for determination of PPCPs in aquatic samples. In preparation for submittal to *Chemosphere*.

Brass, D., Hoyle, G. W., Poovey, H.G., Liu, J. -Y., and Brody, A.R. Reduced TNF- α and TGF- β 1 expression in the lungs of inbred mice that fail to develop fibroproliferative lesions consequent to asbestos exposure. *Am. J. Pathol.* **154**:853-862, 1999.

Burow, M.E., Weldon, C.B., Chiang, T-C., Tang, Y., Collins-Burow B.M., Rolfe, K., Li, S., McLachlan, J.A., Beckman, B.S. Differences in protein kinase C and estrogen receptor α , β expression and signaling correlate with apoptotic sensitivity of MCF-7 breast cancer cell variants. *Int. J. Oncol.* **16**: 1179-1187, (2000).

Burow, M.E., Boue, S.B., Collins-Burow, B.M., Melnik, L.I., Duong, B.N., Li, S.F., Wiese, T., Cleavland, E., McLachlan J.A. Phytochemical glyceollins, isolated from soy, mediate anti-hormonal effects through estrogen receptor alpha and beta. *J. Clin. Endocrinol. and Metabolism* **86(4)**, 1750-1758, (2001).

Burow, M.E., Weldon, C.B., Tang Y., McLachlan, J.A., Beckman, B.S. Oestrogen-mediated suppression of TNF-induced apoptosis in MCF-7 cells: subversion of Bcl-2 by anti-oestrogens. *J. Steroid Biochem. & Mol. Biol.* **78(5)**: 409-418, (2001).

Burow, M.E., Collins-Burow, B.M., Frigo, D.E., Weldon, C.B., Elliot, S., Alam, J., McLachlan, J.A. Antiestrogenic activity of flavonoid phytochemicals mediated via c-jun N-terminal protein kinase and p38, Mitogen-activated protein kinase pathways. Isoform specific antagonism of estrogen receptor alpha. In preparation for submission to *Endocrinology*.

Chen, N., T. Bianchi, and J. Bland. Novel sediment pigment biomarkers as indicators of grazing on the Louisiana shelf: The application of high-performance liquid chromatography-mass spectrometry (HPLC-MS) techniques. *Marine Chemistry*. In Preparation.

Chen, N., T. Bianchi, **B. McKee**, and J. Bland. Fate of chlorophyll-a in the lower Mississippi River and Louisiana shelf: Effects of differential sedimentation and redox. *Geochimica Comochimica Acta*. In Preparation

Chen, N., T. Bianchi, **B. McKee**, and J. Bland. Historical Trends of Hypoxia on the Louisiana Shelf: the Application of Pigments as Biomarkers. *Organic Geochemistry*. 32 (4): 543-561 2001

Collins-Burow, B.M., **Burow, M.E.,** Duong, B.N., McLachlan, J.A. Estrogenic and antiestrogenic activities of flavonoid phytochemicals through estrogen receptor binding-dependent and -independent mechanisms. *Nutrition and Cancer*. **38(2)**, 229-244 (2000).

Collins-Burow, B.M., **Burow, M.E.,** Weldon, C.B., McLachlan, J.A. Induction of apoptosis by anti-estrogenic phytochemicals in breast carcinoma cells. Manuscript in preparation for submission.

Corbett, R., **B. McKee** and D. Duncan. The fate of particle-reactive radionuclides on a river-dominated margin: The Mississippi River and adjacent shelf. *Geochimica et Cosmochimica Acta*. In Preparation

Dagg, M.J. and H. Liu. Grazing by the microzooplankton and mesozooplankton communities in the vicinity of the Mississippi River plume during spring and fall 2000. *Marine Ecology Progress Series*. In Preparation

Delehanty, J.B., H. Yu, and **D.A. Blake** (2001) Lysine 58 in the heavy chain of a monoclonal antibody specific for chelated complexes of lead is important for antigen recognition. *J. Biol Chem.*, submitted.

Delehanty, J.B., M. Khosraviani, R.C. Blake II, H. Yu, and **D.A. Blake** (2001) Recognition of Pb (II)-chelate complexes by a monoclonal antibody and its F_{ab} fragment, *Bioconjugate Chem.*, submitted.

Feng, Z.-G. and **Michaelides, E. E.**, "Drag coefficients of viscous spheres at intermediate and high Reynolds numbers," *J. Fluids Eng.*, in print, 2001.

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APPENDIX C.

USEABLE TECHNOLOGIES

Summary of Useable Technologies

Environmental Signals and Sensors

BLAKE, D.

1. **Title of technology product:** Immunosensor for deployment in AUV;
Recombinant antibodies for environmental analysis
2. **Description of technology product:** This antibody-based biosensor will be able to automatically collect and analyze 5 separate samples after installation in an autonomous underwater vehicle or immobilized buoy (EARS);
3. **Utility/benefit/ROI/payoff of technology product:** A self-contained, automated immunosensor will have the capability to detect very low concentrations of environmental contaminants and/or chemical and biological weapons in surface waters.
4. **Timeline (demonstration, validation, completion, etc.):** An assay that detects nanomolar levels of EDTA, the first analyte to be developed for this instrument, has already been established on the KinExA 3000 research instrument. Performance of the prototype assay in Mississippi River water will be assessed in the next 3 months. Transfer of the assay to the immunosensor will begin when Sapidyne completes the assembly of the instrument.
5. **Partners (academia, industry, labs/centers, federal agency, etc.):** Sapidyne Instruments (Boise, ID) is constructing the immunosensor and our laboratory is working closely with them to coordinate the development of biological reagents with the development of the instrument. The Blake laboratories also have strong ties with Dr. Fran Ligler's laboratory at the Naval Research Laboratory in Washington D.C. James Delehanty, who recently received his Ph.D. in Diane Blake's laboratory, is now an NRC fellow in Dr. Ligler's laboratory.
6. **Patents (applied for and issued):** J.B. Delehanty and D.A. Blake "Recombinant antibodies that bind to metal-chelate complexes", provisional patent application filed 3/29/2001.

Ecosystem Monitoring and Assessment

MEFFERT

1. **Title of technology product**
Integrated autonomous immunosensor and autonomous underwater vehicle system.
2. **Description of technology product**
Facilitated interactions to promote coordinated research between Tulane and Xavier investigators; Naval Oceanographic Office, Stennis, MS; and private sector (COTS Technology, LLC and Sapidyne Instruments) for joint research on biosensor coupling with autonomous underwater vehicle navigation enhancement and "intelligent" programming for long-term deployment, accuracy, and self-regulation of biosensors in aquatic environments.
3. **Utility/benefit/ROI/payoff of technology product**
Enhanced real-time biosensor deployment for environmental compliance and, ultimately biologic warfare detection.
4. **Timeline** (demonstration, validation, completion, etc.)
AUV navigation study to be completed in January 2001. Biosensor to be deployed on AUV or stationary buoys in fall 2001.
5. **Partners** (academia, industry, labs/centers, federal agency, etc.)
Naval Oceanographic Office
Tulane and Xavier Universities
Sapidyne Instruments, Boise, Idaho
COTS Technology, LLC, New Orleans, LA
Woods Hole Oceanographic Institute, Woods Hole, Massachusetts
6. **Patents** (applied for and issued)
Patents will be applied for by partners

APPENDIX D.

INTELLECTUAL DEVELOPMENT

Intellectual Development

<u>Student Name</u>	<u>Level</u>	<u>Institution</u>	<u>Mentor</u>
Cong-Tran, Sabine	Post-doctoral	Tulane University	Efstathios Michaelides, Ph.D.
Feng, Zhi-Gang	Post-doctoral	Tulane University	Efstathios Michaelides, Ph.D.
Huang, Taisheng	Post-doctoral	Tulane University	Chao-Jun Li, Ph.D.
Xia, Wen-Sheng	Post-doctoral	Tulane University	Chao-Jun Li, Ph.D.
Zhang, Wen-Chun	Post-doctoral	Tulane University	Chao-Jun Li, Ph.D.
Basavapathruni, Radha	Graduate	Tulane University	Thomas Bishop, Ph.D.
Bernard, John	Graduate	Tulane University	Doug Meffert, Ph.D.
Carlson, John	Graduate	Tulane University	Scott Michael, Ph.D.
Collins-Burow, Bridgette M.	Graduate	Tulane University	Matthew Burow, Ph.D.
Conard, Craig	Graduate	Tulane University	Dawn Wesson, Ph.D.
Costellio, Chris	Graduate	Tulane University	Chao-Jun Li, Ph.D.
Craig, Lorenzo	Graduate	Tulane University	Elia Eschenazi, Ph.D.*
Delehanty, James B.	Graduate	Tulane University	Diane Blake, Ph.D.
Frigo, Daniel E.	Graduate	Tulane University	Matthew Burow, Ph.D.
Galler, John	Graduate	Tulane University	Bernard J. Coakley, Ph.D.
Gibbs, Monique	Graduate	Xavier University	Elia Eschenazi, Ph.D.*
Hunt, Nicole	Graduate	Tulane University	Thomas Bishop, Ph.D.
Kriegel, Alison M.	Graduate	Tulane University	Diane Blake, Ph.D.
Martin, Jim	Graduate	Tulane University	Efstathios Michaelides, Ph.D.
Mitchell, Kameron A.	Graduate	Tulane University	Matthew Burow, Ph.D.
Morales, Maria	Graduate	Tulane University	Dawn Wesson, Ph.D.
Nguyen, Summer	Graduate	Tulane University	Dawn Wesson, Ph.D.
Niyendra, Swirvine	Graduate	Tulane University	Efstathios Michaelides, Ph.D.
Ocampo, Clara	Graduate	Tulane University	Dawn Wesson, Ph.D.
Peel, Bethany	Graduate	Tulane University	Dawn Wesson, Ph.D.
Sadaka, Mark	Graduate	Tulane University	Thomas Bishop, Ph.D.
Shelby, Bryan	Graduate	Tulane University	Dawn Wesson, Ph.D.
Von Burg, Andrea	Graduate	Tulane University	Dawn Wesson, Ph.D.
Wang, Xian-Yong	Graduate	Tulane University	Chao-Jun Li, Ph.D.
Wyche, Melodie	Graduate	Tulane University	Elia Eschenazi, Ph.D.*
Xu, Xujia	Graduate	Tulane University	Efstathios Michaelides, Ph.D.
Xyuan, Xiaojing	Graduate	Tulane University	Fernando Figueroa, Ph.D.

* Tulane/Xavier collaborative mentoring program 3+2+2 leading to a master's degree in Engineering.

Intellectual Development

<u>Student Name</u>	<u>Level</u>	<u>Institution</u>	<u>Mentor</u>
Au, Dan	Undergraduate	Tulane University	Christine Murphey, MSW
Baird, Breck	Undergraduate	Tulane University	Christine Murphey, MSW
Carter, Junaia	Undergraduate	Xavier University	Thomas Bishop, Ph.D.
Casas, Marcela	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Cervenka, Alexandra	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Christine, Tanner	Undergraduate	Tulane University	Christine Murphey, MSW
Daniels, Rebecca	Undergraduate	Tulane University	Christine Murphey, MSW
Davis-Molier, Shakia	Undergraduate	Xavier University	Thomas Bishop, Ph.D.
Devery, Maureen	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Ercuemen, Ayse	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Everett, Mike	Undergraduate	Tulane University	Christine Murphey, MSW
Franke, Betsy	Undergraduate	Tulane University	Christine Murphey, MSW
Fruchter, Dan	Undergraduate	Tulane University	Christine Murphey, MSW
Gamble, Lena	Undergraduate	Xavier University	Thomas Bishop, Ph.D.
Hall, Kendria	Undergraduate	Xavier University	Thomas Bishop, Ph.D.
Hills, Jasmine	Undergraduate	Tulane University	Doug Meffert, Ph.D.
Huthmaker, Mark	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Jones, Cecily	Undergraduate	Xavier University	Scott Michael, Ph.D.
Karam, Jennifer	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Kemp, Katie	Undergraduate	Tulane University	Christine Murphey, MSW
Kramer, Spencer	Undergraduate	Tulane University	Doug Meffert, Ph.D.
Laflour, J.	Undergraduate	Tulane University	Chao-Jun Li, Ph.D.
Lerhner, Erin	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Lourido, Sebastin	Undergraduate	Tulane University	Scott Michael, Ph.D.
Lu, Lu	Undergraduate	Tulane University	David J. Sailor, Ph.D.
Ma, Yuebo	Undergraduate	Tulane University	Fernando Figueroa, Ph.D.
Mellad, Jason	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Moczygemba, Shelly	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Nelson, Rachel	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Pang, Matthew	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Paul, Alana	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Reemtsma, Helge	Undergraduate	Tulane University	Glen R. Boyd, Ph.D., P.E.
Singh, Yojna	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Smeby, Kristen	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Tanner, Shannon	Undergraduate	Tulane University	Christine Murphey, MSW
Thanos, Nikki	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Thompson, Gretchen	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Vernon, Melissa	Undergraduate	Tulane University	Christine Murphey, MSW
Vincent, Shawn	Undergraduate	Loyola University	Scott Michael, Ph.D.
Wiley, Seth	Undergraduate	Tulane University	Doug Meffert, Ph.D.
Williams, Eman	Undergraduate	Tulane University	Elizabeth Davey, Ph.D.
Wysocki, Laura	Undergraduate	Tulane University	Brent McKee, Ph.D.
Dunson, Katrina	SPRITE	Tulane University	Cynthia Gullledge
Johnson, Tametra	SPRITE	Tulane University	Michael Schurr
Jones, Cecily	SPRITE	Tulane University	Scott Michael
Jupiter, Kendra	SPRITE	Tulane University	Pernilla Wittung-Stafshede
Smith, Randi	SPRITE	Tulane University	Diane Blake
Williams, Danielle	SPRITE	Tulane University	Paul Columbo

APPENDIX E.

HISTORICAL DOCUMENTS

Office of Naval Research



800 N. Quincy St., Arlington, VA 22217-5660

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As published in the Commerce Business Daily on July 31, 1998

Solicitation Number: {98-019}

Due Date: {within one year of publication date}

Classification: A

Type: Procurement

Agency:

{Agency Address}
Office of Naval Research {Agency Address}
800 North Quincy Street {Agency Address}
Arlington, VA 22217-5660

Title: {Long Range Scientific and Technology Research Projects}

Point of Contact: {Beverly Harris ONR, OOST (703) 696-5419}

Synopsis:

{BAA 98-019 The Office of Naval Research (ONR) is interested in receiving proposals for long-range science and technology research projects which offer potential for advancement and improvement of naval operations. This notice constitutes ONR's Broad Agency Announcement (BAA) as contemplated in FAR 6.102(d)(2). Readers should note that this is an announcement to declare ONR's broad role in competitive funding of meritorious research across a spectrum of science and engineering disciplines. No request for proposal (RFP), solicitation or other announcement of this opportunity will be made. This announcement will be open for one year from date of publication or until replaced by a successor BAA. Proposals may be submitted any time during this period. Awards may take the form of contracts, grants, cooperative agreements, or other transactions. Proposal submission is not restricted in any way to any particular entity. Historically Black Colleges and Universities, Minority Institutions, Tribal Colleges and Universities, and small, small disadvantaged, and women owned small businesses are encouraged to participate. Before preparing proposals, potential offerors are encouraged to contact the ONR Program Officer whose program best matches the offeror's field of interest as listed in the Science and Technology section of the ONR Home Page accessible through World Wide Web at <http://www.onr.navy.mil>. OFFERORS MUST STATE IN THEIR PROPOSAL (PREFERABLY ON THE COVER PAGE) THAT IT IS SUBMITTED IN RESPONSE TO THIS BAA. Proposals should also be accompanied by a completed certification package which can be accessed on the ONR Home Page at "[How to Submit a Proposal](#)". For grant proposals (normally submitted by universities) the certification package is entitled "[Certifications and submittal statement document](#)". For contract proposals (normally submitted by commercial and non-profit contractors) the certification package is entitled "[Representations and Certifications](#)". Award decisions will be based on a competitive selection of proposals resulting from a scientific/technical review. Evaluations will be conducted using the following evaluation criteria: (1) overall scientific, technical and/or socio-economic merits of the proposal; (2) potential naval relevance and contributions of the effort to the agency's specific mission; (3) the offeror's capabilities, related experience, facilities, techniques or unique combinations of these which are integral factors for achieving the proposal objectives; (4) the qualifications, capabilities and experience of the proposed Principal Investigator, team leader and key personnel who are critical in achieving the proposal objectives; (5) realism of the proposed cost and availability of funds. For awards made as contracts, the socio-economic merits of each proposal will be evaluated based on the commitment to provide meaningful subcontracting opportunities for Small Business, Small Disadvantaged Business, Woman-Owned Small Business Concerns, Historically Black Colleges and Universities, Minority Institutions, and Tribal Colleges and Universities. The standard industrial classification code is 8731 with the small business size standard of 500 employees. In addition, contract proposals that exceed \$500,000 submitted by all but small business, must be accompanied by a Small, Small Disadvantaged and Women-Owned Small Business Subcontracting Plan in accordance with FAR 52.219-9.} *****

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[Employment](#) | [Business Opportunities](#)
[Education Opportunities](#) | [Home](#) | [Search](#)



DEPARTMENT OF THE NAVY
OFFICE OF NAVAL RESEARCH
800 NORTH QUINCY STREET
ARLINGTON, VA 22217-5680

IN REPLY REFER TO
242:SBM

APR 23 1999

TULANE UNIVERSITY
OFFICE OF RESEARCH AND PROJECT ADMINISTRATION
327 GIBSON HALL
NEW ORLEANS, LA 70118-5698

Gentlemen:

Enclosed for your retention is one copy of Grant Number N00014-99-1-0763 which I have signed for the Government. The grant document does not require your signature.

Please acknowledge receipt of this grant by promptly signing and returning the enclosed copy of this transmittal letter to this office to the attention of ONR 242:SBM. Keep this original letter for your records.

In the event of any disagreement with the grant provisions, you must notify this office within thirty (30) days of the date of this letter. If you have any questions, please contact Sadie B. Marshall by telephone on (703) 696-2577.

Sincerely,

TERRY W. YOUNG
Grants Officer

Grants Officer

Enclosure

Acknowledgement of Receipt

By: Janeth A. Wilks Date: 5/3/99

Office of Naval Research
ONR 242:Sadie B. Marshall (703) 696-2577
Ballston Centre Tower One
800 North Quincy Street
Arlington, VA 22217-5660

GRANT NO:	N00014-99-1-0763
PR NUMBER:	99PR06417-00
P.O. CODE:	335
DISBURSING CODE:	N68892
AGO CODE:	N66020
CAGE CODE:	0F7R1
PI:	John McLachlan

CFDA No: 12.300

RESEARCH GRANT

DUPLICATE

GRANTEE: TULANE UNIVERSITY
OFFICE OF RESEARCH AND PROJECT
ADMINISTRATION
327 GIBSON HALL
NEW ORLEANS, LA 70118-5698

APPROPRIATION: See attached *Financial Accounting Data Sheet(s)*.

TOTAL AMOUNT OF GRANT: \$4,660,000.00

AUTHORITY: 10 U.S.C. 2358 as amended, and 31 U.S.C. 6304.

GRANT SCHEDULE

GRANT PURPOSE: The purpose of this grant is to support research in bioenvironmental science. The research shall be in accordance with Grantee's proposal dated 24 MAR 99, as revised 09 APR 99, entitled, "Integrated Bioenvironmental Hazards Research Program", incorporated herein by reference.

PERIOD: The grant is for the period 01 MAY 99 through 30 APR 01.

TERMS AND CONDITIONS: This grant is subject to the Office of Naval Research General Terms and Conditions for Educational Institutions, Nonprofit Organizations, and State and Local Governments dated SEPTEMBER 1998, which are incorporated herein by reference and listed by title in Exhibit A, and to any special terms and conditions contained in this grant schedule.

PRINCIPAL INVESTIGATOR: The Principal Investigator, John McLachlan, shall be continuously responsible for the research project. The Grantee agrees to obtain the written approval of the Grantor before changing the Principal Investigator.

PROGRAM OFFICER: The Program Officer representing the United States Government is Harold E. Guard, ONR 335, Office of Naval Research, Ballston Centre Tower One, 800 North Quincy Street, Arlington, VA 22217-5660. (703) 696-4311

ADMINISTRATIVE GRANTS OFFICER: The Administrative Grants Officer (AGO) for this grant is:

OFFICE OF NAVAL RESEARCH REGIONAL OFFICE ATLANTA
SUITE 4R15
100 ALABAMA STREET NW
ATLANTA, GA 30303-3104

UNEXPENDED FUNDS AND EARNED INTEREST:

a. At the end of the grant period, any unexpended balances of funds provided by the grant shall be remitted to the Office of Naval Research at the address given in the above paragraph titled "Administrative Grants Officer," by check made payable to the Office of Naval Research.

b. Any interest earned by grant funds on deposit shall be remitted annually to the Department of Health and Human Services, Payment Management System, P.O. Box 6021, Rockville, MD 20852, by check made payable to the Department of Health and Human Services.

REPORTS AND REPORT DISTRIBUTION:

Reports shall be furnished as specified in Attachment Number 1, entitled, 'Reports and Report Distribution,' of this grant. The Grantee shall include a completed 'Report Documentation Page' (SF-298) as the last page of each copy of every scientific and technical report prepared under this grant. The cognizant Grants Administrator at the address given in the 'Administrative Grants Officer' paragraph above will provide assistance to the Grantee in obtaining the required form.

The form contains instructions for preparation. However, Block 12a of the form should be completed with the following distribution/availability statement: APPROVED FOR PUBLIC RELEASE. If the Grantee does not agree with that distribution/availability, the Grantee should contact the cognizant ONR Grants Administrator. The form is not required when submitting administrative type reports and managerial (status) reports.

PAYMENTS: Upon acceptance of the terms and conditions of this grant, the Grantee may submit a request for reimbursement using an original Standard Form 270 (SF-270), plus two copies, to the AGO. Requests for payment may not exceed the amount obligated to this grant. If advance payments are desired, the grantee may submit SF-270 in accordance with OMB Circular A-110. Section 11 of the SF-270 must be completed in its entirety. At the discretion of the AGO, a payment schedule may be required of the Grantee.

MILITARY RECRUITING ON CAMPUS (DODGARS 23.1, Interim Rule, 60 FR 4544-45, 24 January 1995): As a condition for receipt of funds available to the Department of Defense (DOD) under this award, the recipient agrees that it is not an institution that has a policy of denying, and that it is not an institution that effectively prevents, the Secretary of Defense from obtaining for military recruiting purposes: (A) entry to campuses or access to students on campuses; or (B) access to directory information pertaining to students. If the recipient is determined, using procedures established by the Secretary of Defense to implement section 558 of Public Law 103-337 (1994), to be such an institution during the period of performance of this agreement, and therefore to be in breach of this clause, the Government will cease all payments of DOD funds under this agreement and all other DOD grants and cooperative agreements, and it may suspend or terminate such grants and agreements unilaterally for material failure to comply with the terms and conditions of award.

This grant is issued under the Authority of 10 U.S.C. 2358 as amended, and 31 U.S.C. 6304.

UNITED STATES OF AMERICA

Office of Naval Research

BY

Grants Officer

DATE

4/22/99

TERRY W. YOUNG
Grants Officer

ATTACHMENT NUMBER 1

REPORTS AND REPORT DISTRIBUTION

REPORT TYPES

- (a) Performance (Technical) Report(s) (Include letter report(s)) Frequency: Annual*
- (b) Final Technical Report, issued at completion of Grant. *
NOTE: Final Technical Reports must have a SF-298 accompanying them. Unless otherwise stated in the grant, complete Block 12a. of the SF-298: "Approved for Public Release; distribution is Unlimited."
- (c) Final Financial Status Report (SF 269)
- (d) Final Patent Report (DD 882)

REPORTS DISTRIBUTION		
ADDRESSEES	REPORT TYPES	NUMBER OF COPIES
Office of Naval Research Program Officer Harold E. Guard ONR 335 Ballston Centre Tower One 800 North Quincy Street Arlington, VA 22217-5660	(a) & (b) w/(SF-298's)	3
Administrative Grants Officer OFFICE OF NAVAL RESEARCH REGIONAL OFFICE ATLANTA SUITE 4R15 100 ALABAMA STREET NW ATLANTA, GA 30303-3104	(c), (d) & SF-298's only for (a) & (b)	1
Director, Naval Research Laboratory Attn: Code 5227 4555 Overlook Drive Washington, DC 20375-5326	(a) & (b) w/(SF-298's)	1
Defense Technical Information Center 8725 John J. Kingman Road STE 0944 Ft. Belvoir, VA 22060-6218	(a) & (b) w/(SF-298's)	2
Office of Naval Research Attn: ONR 00CC1 Ballston Centre Tower One 800 North Quincy Street Arlington, VA 22217-5660	(d)	1

If the Program Officer directs, the Grantee shall make additional distribution of technical reports in accordance with a supplemental distribution list provided by the Program Officer. The supplemental distribution list shall not exceed 250 addresses.

* For report types (a) and (b), send only a copy of the transmittal letter to the Administrative Contracting Officer; do not send actual reports to the Administrative Contracting Officer.

EXHIBIT A

The following terms and conditions are incorporated herein by reference with the same force and effect as if they were given in full text. Upon request, the Administrative Grants Officer named herein will make their full text available.

OFFICE OF NAVAL RESEARCH

GRANT GENERAL TERMS AND CONDITIONS
FOR EDUCATIONAL INSTITUTIONS, NONPROFIT
ORGANIZATIONS, AND STATE AND LOCAL GOVERNMENT

ARTICLE

1. Federal Requirements
2. Order of Precedence
3. Administration and Cost Principles
4. Research Responsibility
5. Amendment of Grant
6. University Grantees
7. Principal Investigator
8. Publications
9. Acknowledgment of Sponsorship
10. Grantee-Acquired Property
11. Patent Rights
12. Rights in Technical Data and Computer Software
13. Human Subjects
14. Animal Welfare
15. Research Involving Recombinant DNA Molecules
16. Reserved
17. Activities Abroad
18. Nondiscrimination
19. Clean Air and Water
20. U.S. Flag Air Carriers
21. Security
22. Subawards and Contracts/Subcontracts
23. New Restrictions on Lobbying

1 PR NUMBER
00PR06417-00

3 MOD (CRITICAL)

2 SPIN (CRITICAL)

CONTRACT NUMBER (CRITICAL)
N000148910703

1 LINE OF ACCOUNTING

2 APPROPRIATION (CRITICAL)

3 SUBHEAD (CRITICAL)

4 OBJ CLS

5 PARM

6 RFM

7 SA

8 AAA (CRITICAL)

9 IT

10 PAA

11 K

12 PROJ UNIT

13 MCC

14 PDI & SUE

15 AMOUNT (CRITICAL)

16 NAVY INTERNAL USE ONLY REF DOG/ACRN

17 PR06SPR06417-00 FRC35C1

18 4,660,000.00

19 3010

20 02601

21 000000

22 2D

23 068342

24 0

25 333

26 RA

27 000

28 W224

29 1791319

30 AA

31 4,660,000.00

32 4,660,000.00

33 PAGE TOTAL

34 GRAND TOTAL

35 \$4,660,000.00

36 \$4,660,000.00

37 COMPTROLLER APPROVAL:

38 FOR FISCAL DATA AND SIGNATURE

39 BY

40 REVIEWED

41 DATE:

42 FOR COMPTROLLER ONR CONTRACT

43 PREPARED AUTHORIZED BY:

44 DATE:

45 FA01

OFFICE OF NAVAL RESEARCH
GRANT GENERAL TERMS AND CONDITIONS
FOR EDUCATIONAL INSTITUTIONS, NONPROFIT
ORGANIZATIONS, AND STATE AND LOCAL GOVERNMENT

September 1998 Revised

ARTICLE

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- 20. U.S. Flag Air Carriers
- 21. Security
- 22. Subawards and Contracts/Subcontracts
- 23. New Restrictions on Lobbying

ONR General Grant Terms and Conditions

September 1998 Revised

1. Federal Requirements

This Grant is subject to the laws and regulations of the United States. If any statute expressly prescribes policies or specific requirements that differ from the requirements, standards, provisions, or terms and conditions of this Grant, the provisions of the statute shall govern.

2. Order of Precedence

Any inconsistency or conflict in the terms and conditions specified in this Grant shall be resolved according to the following order of precedence:

- a. The Federal statute authorizing this award, or any other Federal statutes directly affecting performance of this Grant.
- b. These General Terms and Conditions.
- c. Other terms and conditions contained within the Grant and any attached schedules.

3. Administration and Cost Principles

Applicable to this Grant, and incorporated herein by reference, are the requirements, standards, and provisions of the appropriate OMB Circulars and attachments thereto, as revised as of the effective date of this Grant, listed below. For purposes of this paragraph, the term "appropriate" is determined by the organizational nature of the Grantee (educational institution, nonprofit organization, state or local government).

- (a) A-110, "Uniform Administrative Requirements for Grants and Agreements with Institutions of Higher Education, Hospitals, and Other Nonprofit Organizations"
- (b) A-21 "Cost Principles for Educational Institutions"
- (c) A-122 "Cost Principles for Nonprofit Organizations"
- (d) A-87 "Cost Principles for State and Local Governments"
- (e) A-102 "Grants and Cooperative Agreements with State and Local Governments"

(f) A-133 "Audits of Institutions of Higher Learning and Other Non-Profit Institutions"

4. Research Responsibility

The Grantee has full responsibility for the conduct of the research activity supported by this Grant, in accordance with the Grantee's proposal, and the terms and conditions specified in this Grant.

Grantees are encouraged to suggest or propose to discontinue or modify unpromising lines of investigation or to explore interesting leads which may appear during the development of the research. However, they must consult the Program Officer through the Administrative Grants Officer before significantly deviating from the objectives or overall program of the research originally proposed.

5. Amendment of Grant

The only method by which this Grant can be amended is by a formal, written amendment signed by either the Grants Officer or the Administrative Grants Officer. No other communications, whether oral or in writing, are valid.

6. University Grantees

a. Prior Approvals. All prior approvals required by OMB Circulars A-21 and A-110 are waived hereby except for the following:

(1) Change of scope or objectives as required by Article 4 of these Terms and Conditions entitled "Research Responsibility".

(2) Change of key personnel as required by Article 7 of these Terms and Conditions entitled "Principal Investigator".

(3) Extension of the expiration period of this Grant.

b. Preaward Costs,

(1) Grantees may incur preaward costs of up to ninety (90) days prior to the effective date of the Grant award.

(2) Preaward costs as incurred by the Grantee must be necessary for the effective and economical conduct of the project, and the costs must be otherwise allowable in accordance with the appropriate cost principles.

(3) Any preaward costs are made at the Grantee's risk. The incurring of preaward costs by the Grantee does not impose any obligation on the Office of Naval Research in the absence of appropriations, if an award is not subsequently made, or if an award is made for a lesser amount than the Grantee expected.

c. Unexpended Balances

In the absence of any specific notice to the contrary, Grantees are authorized to carry forward

unexpended balances of funds received to subsequent funding periods.

7. Principal Investigator

Support for the project may not continue without the active direction of the Principal Investigator (PI) approved for, and identified in, this Grant. If the approved PI (1) severs his or her connection with the Grantee or (2) otherwise relinquishes active direction of the project either permanently or for a significant length of time (three months or more), then the Grantee must either:

- (a) appoint a replacement PI with the approval of the Program Officer, or
- (b) relinquish the Grant, in which case the Grant shall be terminated in accordance with OMB Circular A-110, subpart C, paragraph 61, entitled "Termination".

8. Publications

Publication of results of the research project in appropriate professional journals is encouraged as an important method of recording and reporting scientific information. One copy of each paper planned for publication will be submitted to the Program Officer simultaneously with its submission for publication. Following publication, copies of published papers shall be submitted to the Program Officer.

9. Acknowledgment of Sponsorship

(a) The Grantee agrees that in the release of information relating to this Grant, such release shall include a statement to the effect that the project or effort depicted was or is sponsored by the Department of the Navy, Office of Naval Research, and that the content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsement should be inferred.

(b) For the purpose of this article, information includes new releases, articles, manuscripts, brochures, advertisements, still and motion pictures, speeches, trade association proceedings, symposia, etc.

(c) Nothing in the foregoing shall affect compliance with the requirements of the clause entitled "Security".

10. Grantee-Acquired Property

Title to all nonexpendable and expendable tangible personal property purchased by the Grantee with grant funds shall be deemed to have vested in the Grantee upon purchase, unless stated otherwise in this Grant schedule, without further obligation to the Government.

11. Patent Rights

Patent rights are as specified in 37 CFR 401 entitled "Rights to Inventions Made by Nonprofit Organizations and Small Business Firms under Government Grants, Contracts, and Cooperative Agreements." The "Standard patent rights clause" at 37 CFR 401.14 is modified at section (f) thereof to include the additional requirements stated in 37 CFR 401.5(f)(1), (2), and (3).

Invention disclosures are to be submitted to the Associate Counsel/Senior ONR Patent Attorney (Code 00CC), Office of Naval Research, Department of the Navy, 800 North Quincy Street, Arlington, Virginia 22217-5660. That individual will represent the Administrative Grants Officer with regard to invention reporting matters arising under this Grant.

12. Rights in Technical Data and Computer Software

The Government will receive unlimited rights in all technical data and unrestricted rights in all computer software resulting directly from the performance of experimental, developmental, or research work which is specified as an element of performance under this Grant or any other Grant, contract or any subcontract made hereunder. Unlimited rights, as used in this clause, means rights to use, duplicate, release or disclose technical data or computer software in whole or in part, in any manner and for any purpose whatsoever, and to have or permit others to do so.

Such unlimited rights will apply unless the Grantee and the Grantor have negotiated in advance that the Government will receive, for a fixed period of time, less than unlimited rights in data or software generated under the Grant. At the end of such period, the Government shall regain unlimited rights in all Grant data. The Government shall have unlimited rights in any preexisting data furnished by the grantee unless an appropriate legend is affixed to the data by the Grantee limiting the Government's rights to such pre-Grant data or software. Cf. 48 Code of Federal Regulations '252.227-7013.

13. Human Subjects

Grant funds may NOT be used for research that uses uninformed or non-voluntary humans as experimental subjects. The Grantee is responsible for the protection of the rights and welfare of any human subjects involved in research, development, and related activities supported by this Grant. The Grantee agrees to comply, as appropriate, with the following directives and regulations (or their successors) which are incorporated into the Grant by reference:

- a) DoD Directive 3216.2, "Protection of Human Subjects in DoD Supported Research", 7 January 1983, as amended.
- b) DHHS Regulations, "Protection of Human Subjects" (45 Code of Federal Regulations, Part 46) of 18 & 28 June 1991, as amended; and,
- c) FDA Regulations (21 Code of Federal Regulations, subchapters A, D, and H)

14. Animal Welfare

Any Grantee performing research on warm blooded vertebrate animals shall comply with the Laboratory Animal Welfare Act of 1966, as amended, (7 U.S.C. 2131 et seq.), and the regulations promulgated thereunder by the Secretary of Agriculture (9 CFR, Subchapter A, Parts 1 through 4) pertaining to the care, handling, and treatment of vertebrate animals held or used for research, teaching, or other activities supported by Federal awards. In addition, the Grantee shall comply with the provisions of DoD Directive 3216.1 as implemented by SECNAVINST 3900.38b, "The Use of Animals in DoD Programs", 1 June 1984. The Grantee shall specifically address paragraph 10 of SECNAVINST 3900.38B in reference to written assurance statements and clause 52.235-7002 of the DoD Federal Acquisition Regulations Supplement. The Grantee is also expected to ensure that the guidelines described in DHHS Publication No. (NIH) 85-23, "Guide for the Care and Use of

Laboratory Animals," are followed and to comply with the "Government Principles for the Utilization and Care of Vertebrate Animals Used In Testing, Research, and Training" (included as an Appendix to the NIH Guide).

15. Research Involving Recombinant DNA Molecules

Any Grantee performing research involving recombinant DNA molecules and/or organisms and viruses containing recombinant DNA molecules agrees by acceptance of this award to comply with the National Institutes of Health "Guidelines for Research Involving Recombinant DNA Molecules", Nov 1984 (49 FR 46266-46291) or such later revision of those guidelines as may be published in the Federal Register.

16. Reserved

17. Activities Abroad

The Grantee shall assure that project activities carried on outside the United States are coordinated as necessary with appropriate Government authorities and that appropriate licenses, permits, or approvals are obtained prior to undertaking proposed activities. The awarding agency does not assume responsibility for Grantee compliance with the laws and regulations of the country in which the activity(ies) is (are) to be conducted.

18. Nondiscrimination

Grantees shall comply with the provisions of the Civil Rights Act of 1964 (P.L. 88-352), as amended, and implementing regulations, and the Assurance of Compliance which the Grantee must have on file prior to award of a grant.

This award and any program assisted thereby are also subject to the provisions of Title IX of the Education Amendments of 1972 (P.L. 92-318, 20 USC 1681 et seq.), Section 504 of the Rehabilitation Act of 1973 (29 USC 794), the Age Discrimination Act of 1975 (P.L. 94-135), the implementing regulations issued pursuant thereto, and the Assurance of Compliance which the recipient has made available to the awarding agency.

The recipient shall obtain from each organization that applies to be, or serves as a subrecipient, contractor or subcontractor under this award (for other than the provision of commercially available supplies, materials, equipment, or general support services) an Assurance of Compliance as required by implementing regulations.

19. Clean Air and Water

If the amount of the Grant exceeds \$100,000, the Grantee shall comply with the Clean Air Act (42 U.S.C.1857), as amended; the Water Pollution Control Act (33 U.S.C. 1251), as amended; Executive Order No. 11738; and the related regulations of the Environmental Protection Agency (40 CFR, Part 15). Said regulations, Executive Orders, and Acts are incorporated into this Grant by reference.

20. U. S. Flag Air Carriers

This clause applies if the grant is over \$50,000 and U.S. Government-financed international air

transportation of personnel (and their personal effects) or property will occur in the performance of the grant. Under the Fly America Act, as codified at 49 U.S.C. §1517, grant funds may be used by grantees for air travel on non-U.S. flag air carriers only if service on a U.S. flag air carrier is not available. Regulations implementing the Fly America Act are found in the Federal Travel Regulations, specifically at 41 CFR § 301-3.6(b). Guidelines implementing the Act have been issued periodically by the Comptroller General of the United States under Decision B-138942, most recently in the unpublished decision of March 31, 1981.

21. Security

The Grantee shall not be granted access to classified information under this Grant. If security restrictions should happen to apply to certain aspects of the proposed research, the Grantee will be so informed. In the event that the scientific work under this Grant may need classification, or involve access to or storage of any classified data, the Government shall make a decision on the need to classify, or require such access or storage within 30 days after receipt of a written notice from the Grantee. If the decision is affirmative, the Government shall invoke the clause in OMB Circular A-110, Subpart C, paragraph 61, entitled, "Termination".

22. Subawards and Contracts/Subcontracts

The applicable Federal cost principles for subawards and contracts/subcontracts under this Grant shall be those otherwise applicable to the type of organization receiving the subaward, contract or subcontract. In addition to OMB Circular A-21, the other applicable cost principles are:

- (a) OMB Circular A-122 applicable to other nonprofit organizations, except those specifically exempted by the circular.
- (b) Subpart 31.2 of the Federal Acquisition Regulation (48 CFR 31.2) applicable to commercial firms and those nonprofit organizations specifically exempted from the provisions of OMB Circular A-122.
- (c) OMB Circular A-87 (34 CFR 255) for state and local governments.
- (d) 45 CFR 74, Appendix E, for hospitals.

23. New Restrictions on Lobbying

New Restrictions on Lobbying (32 CFR Part 28, July 1992) apply only if the total amount of the grant exceeds \$100,000.00. In such cases, the lobbying regulations are incorporated herein by reference.



AWARD/ MODIFICATION

3a. ISS: **B**
OFFICE OF NAVAL RESEARCH
BALLSTON CENTRE TOWER ONE
800 NORTH QUINCY STREET
ARLINGTON VA 22217-5660.

1. INSTRUMENT TYPE:
Grant

3b. CFDA:
12.300

2. AUTHORITY:
10 USC 2358, 31 USC 6304

3c. DUNS NUMBER:
008963233

4. AWARD NO.:
N00014-99-1-0763

5. MODIFICATION NO.:
P00001

6. MODIFICATION TYPE:
Renewal

7. PR NO.:
00PR07657-00

PAGE
1 of 5

8. ACTIVITY/AGENCY PROPOSAL NO.:
00034--0003

9. RECIPIENT PROPOSAL NO.:
N/A

10. PROPOSAL DATE:
18-APR-2000

11. ACTIVITY TYPE:
Research

12. PROGRAM TYPE:
N/A

13. ISSUED TO
13a. ADDRESS:

13b. CAGE:
4B966

13c. EDI/EFT NUMBER:

14. REMITTANCE ADDRESS (IF DIFFERENT FROM BLOCK 13):
Same as block #13

TULANE UNIVERSITY
OFFICE OF RESEARCH AND PROJECT
ADMINISTRATION
327 GIBSON HALL
NEW ORLEANS, LA 70118-5698

13d. BUSINESS OFFICE CONTACT:
Fishman, Carla H.

13e. TELEPHONE NUMBER:
(504) 8655272

13f. EMAIL ADDRESS:

15. RESEARCH TITLE AND/OR DESCRIPTION OF PROJECT AND/OR PROPOSAL TITLE:
Integrated Bioenvironmental Hazards Research Program

16. FUNDING	ACTIVITY/AGENCY SHARE	RECIPIENT SHARE	TOTAL
PREVIOUSLY OBLIGATED:	\$4,660,000.00	\$.00	\$4,660,000.00
OBLIGATED BY THIS ACTION:	\$935,000.00	\$.00	\$935,000.00
TOTAL OBLIGATED ON AWARD:	\$5,595,000.00	\$.00	\$5,595,000.00
FUTURE FUNDING:	\$.00	\$.00	\$.00
GRANT TOTAL:	\$5,595,000.00	\$.00	\$5,595,000.00

17. CURRENT FUNDING PERIOD
N/A
THROUGH
N/A

18. PERIOD OF PERFORMANCE
01-MAY-1999
THROUGH
30-APR-2001

19. ACCOUNTING AND APPROPRIATION DATA:
See attached Financial Accounting Data Sheet(s)

20a. PRINCIPAL INVESTIGATOR/RECIPIENT
TECHNICAL REPRESENTATIVE:
(PI) John A. McLachlan

21. TECHNICAL REPRESENTATIVE
21a. NAME: Harold E. Guard

21b. CODE:
ONR 34

21c. ADDRESS:
OFFICE OF NAVAL RESEARCH
BALLSTON CENTRE TOWER ONE
800 NORTH QUINCY STREET
ARLINGTON VA 22217-5660

20b. TELEPHONE NUMBER:
(504) 5856910

20c. EMAIL ADDRESS:

21d. TELEPHONE NUMBER:
(703) 6964311

21e. EMAIL ADDRESS:
guardh@onr.navy.mil

22. AWARDDING OFFICE CONTACT
22a. NAME: Julia M. Gallmon

22b. CODE:
ONR 252

23a. ADMINISTRATIVE OFFICE:

23b. CODE:
N66020

22c. ADDRESS:
OFFICE OF NAVAL RESEARCH
BALLSTON CENTRE TOWER ONE
800 NORTH QUINCY STREET
ARLINGTON VA 22217-5660

OFFICE OF NAVAL RESEARCH REGIONAL OFFICE ATLANTA
100 ALABAMA STREET NW SUITE 4R15
ATLANTA GA 30303-3104
Fax: (404) 5621610

22d. TELEPHONE NUMBER:
(703) 6962609

22e. EMAIL ADDRESS:
gallmoj@onr.navy.mil

24. SUBMIT PAYMENT REQUEST TO:
Same as block #23a

25a. PAYING OFFICE:
DFAS CHARLESTON, SC

25b. CODE:
N68892

26a. PATENT OFFICE:

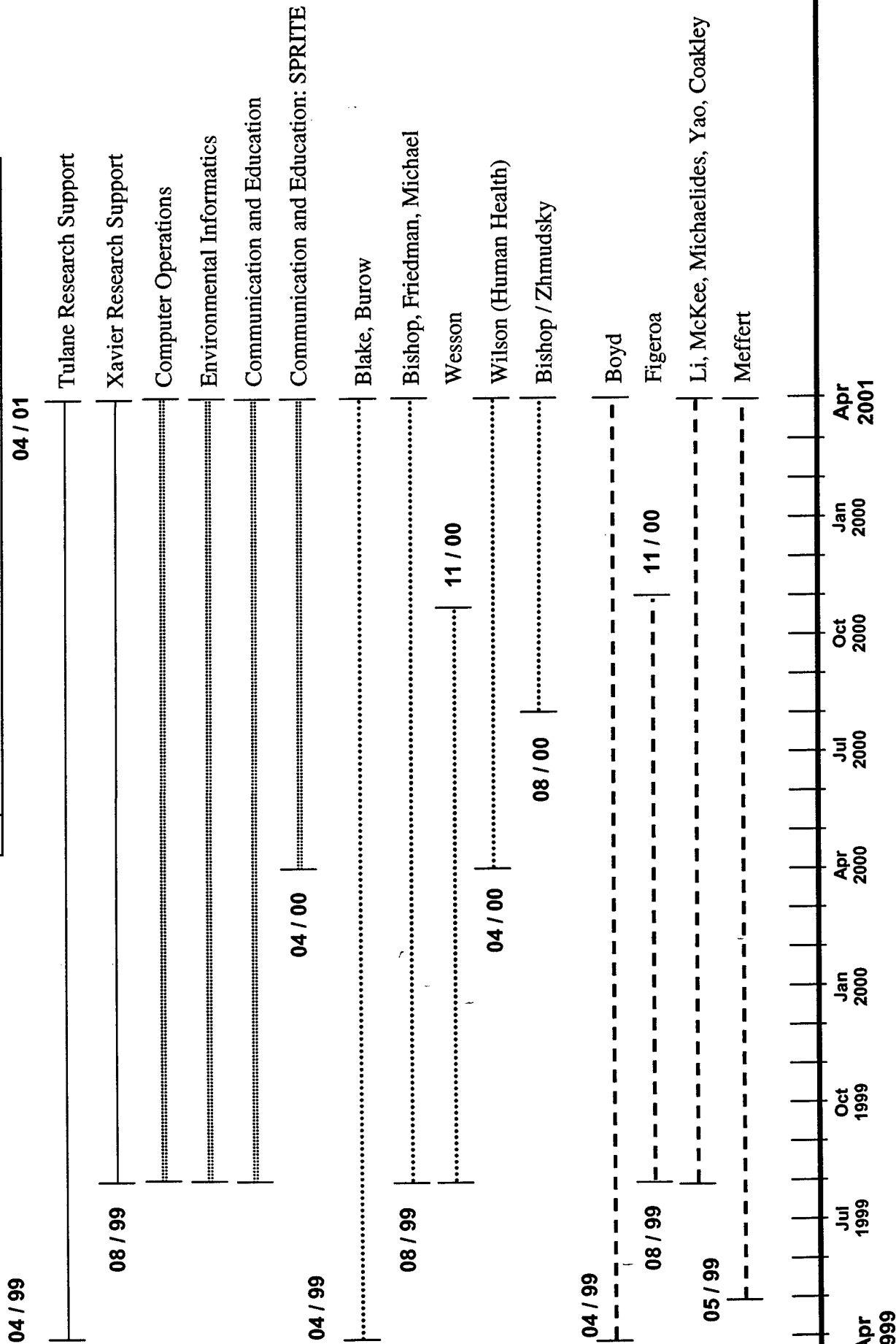
26b. CODE:
N00014

OFFICE OF NAVAL RESEARCH
ATTN: ONR 00CC
BALLSTON CENTRE TOWER ONE
800 NORTH QUINCY STREET
ARLINGTON VA 22217-5660

		AWARD/ MODIFICATION		3a. ISSUED BY: OFFICE OF NAVAL RESEARCH BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660																									
				3b. CFDA: 12.300																									
1. INSTRUMENT TYPE: Grant		2. AUTHORITY: 10 USC 2358, 31 USC 6304		3c. DUNS NUMBER:																									
4. AWARD NO.: N00014-99-1-0763		5. MODIFICATION NO.: P00002		6. MODIFICATION TYPE: Reduction																									
8. ACTIVITY/AGENCY PROPOSAL NO.: N/A		9. RECIPIENT PROPOSAL NO.: N/A		7. PR NO.: 00PR07657-01																									
13. ISSUED TO 13a. ADDRESS: TULANE UNIVERSITY OFFICE OF RESEARCH AND PROJECT ADMINISTRATION 327 GIBSON HALL NEW ORLEANS, LA 70118-5698		13b. CAGE: 4B966		13c. ED/EFT NUMBER:																									
13d. BUSINESS OFFICE CONTACT: Fishman, Carla H.		13e. TELEPHONE NUMBER: (504) 8655272		13f. EMAIL ADDRESS:																									
14. REMITTANCE ADDRESS (IF DIFFERENT FROM BLOCK 13): Same as block #13																													
15. RESEARCH TITLE AND/OR DESCRIPTION OF PROJECT AND/OR PROPOSAL TITLE: Integrated Bioenvironmental Hazards Research Program																													
16. FUNDING				17. CURRENT FUNDING PERIOD																									
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22c. ADDRESS: OFFICE OF NAVAL RESEARCH BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660				23b. CODE: N66020																									
22d. TELEPHONE NUMBER: (703) 6962609		22e. EMAIL ADDRESS: gallmoj@onr.navy.mil																											
24. SUBMIT PAYMENT REQUEST TO: Same as block #23a		25a. PAYING OFFICE: DFAS CHARLESTON, SC		26a. PATENT OFFICE: OFFICE OF NAVAL RESEARCH ATTN: ONR 00CC BALLSTON CENTRE TOWER ONE 800 NORTH QUINCY STREET ARLINGTON VA 22217-5660																									
		25b. CODE: N68892		26b. CODE: N00014																									

ONR 1999 - 2001 Timeline

K E Y	Research Support	Core Support	Environmental Signals and Sensors	Ecosystem Monitoring & Assessment
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Institutional Review Board Approval



Tulane University Health Sciences Center

Office of the
Senior Vice President
for the Health Sciences
1430 Tulane Avenue
New Orleans, Louisiana 70112-2699

Committee on Use of Human Subjects TW36
Tidewater Bldg. Room 830
Phone: (504) 584-2665
Fax: (504) 586-3892

October 25, 2001

EXPEDITED CONTINUING REVIEW APPROVAL

This is to certify that the grant, contract or study entitled:

Identification of DNA Methylation-Related Genes in Human Leiomyomata Uteri (R0088)

Submitted by: **John McLachlan, Ph.D.**

Originally approved on 7/13/98 is an ongoing project that has been re-evaluated in order to provide continuing review.

This expedited approval has been granted in accordance with 45 CFR 46.110.

This action will be presented to the full Committee on Use of Human Subjects at the next regularly scheduled committee meeting on October 25, 2001 to inform the committee members of this action.

HUMAN SUBJECTS – REVIEWED – AT RISK

Continuing approval granted: 10/25/01
This approval expires on: 10/24/02

Ira Friedman, MSN, NP-C, CIM
Chair
Committee on Use of Human Subjects

IF/cl

GENERAL ASSURANCE NUMBER M1260



OCT 03 2001

Materials and Methods

TULANE MEDICAL CENTER
COMMITTEE ON USE
OF HUMAN SUBJECTS

RNA extraction:

RNA will be extracted using the Ultraspec™ RNA isolation kit (Biotecx Lab, TX). In brief, frozen tissues will be smashed and homogenized in RNA isolation solution. Then chloroform will be added. After centrifugation, the colorless upper aqueous phase will be removed to a new tube. An equal volume of isopropanol will be added to precipitate RNA. RNA pellet will be washed with 75% of ethanol and recovered in DEPC-treated water.

Reverse transcription:

Reverse transcription reactions will be carried out using the Gene Amp RNA PCR kit (Perkin Elmer, CA). Manufacturing methods will be followed with modification. The same amount of RNA, 10 µg, will be annealed with 2 µl of 50 µM of oligo dT primers and DEPC treated-water in a final volume of 18 µl. The annealing reaction will be carried out at 70°C for 3 minutes and kept in 4°C until the next extension reactions.

The extension reaction will be carried out by adding annealing mixture to the extension mixture. The extension reaction contains 5 mM MgCl₂, 1X PCR buffer II (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 125 µM of dATP, dGTP, dCTP, dTTP, 40 unit of RNase inhibitors, 5 mM DTT, and 100 units of MuLV Reverse transcriptase and annealing mixture in a final volume of 40 µl. The extension reaction will be held in 42°C for 90 minutes and followed by 70°C for 10 minutes. The cDNA pool will be stored at -70°C for the next PCR reactions.

Quantitative PCR:

PCR reactions will be carried out by adding 10 µl of diluted cDNA into 40 µl of PCR mixture. The PCR mixture contains 1X PCR buffer II (50 mM KCl, 10 mM Tris-HCl, pH 8.3 and 0.001% (w/v) gelatin), 5.5 mM MgCl₂, 0.2 mM dNTP mixture (Roche, CA), 900 nM of each DNMTs primer or 720 nM of each glyceraldehyde phosphate-3-dehydrogenase (GAPDH) primer, 154 nM of each fluorescent probes, 2.5 unit of AmpliTaq Gold DNA polymerase. PCR reactions will be performed on a DNA Thermal

IRB Approval: 10/25/01
Sign By: 10/24/02

To: Institutional Review Board

From: John McLachlan, Ph.D.
Director, Center for Bioenvironmental Research

Re: Changes to Protocol **R0088** since last review, August 8, 2000

Date: Oct. 1, 2001

Enclosed please find the renewal for the project entitled **Identification of DNA Methylation-related Genes in Human Leiomyomata Uteri (R0088)**. Please note the following changes to the Materials and Methods section since the last review on August 8, 2000. The changes outlined in this memo reflect an updated analytical method for RNA extraction and the use of RT-PCR and real-time PCR. The remainder of the protocol that was reviewed on August 8, 2000 remains unchanged.

Thank you.

Materials and Methods

RNA extraction:

RNA will be extracted using the UltraspecTM RNA isolation kit (Biotech Lab, TX). In brief, frozen tissues will be smashed and homogenized in RNA isolation solution. Then chloroform will be added. After centrifugation, the colorless upper aqueous phase will be removed to a new tube. An equal volume of isopropanol will be added to precipitate RNA. RNA pellet will be washed with 75% of ethanol and recovered in DEPC-treated water.

Reverse transcription:

Reverse transcription reactions will be carried out using the Gene Amp RNA PCR kit (Perkin Elmer, CA). Manufacturing methods will be followed with modification. The same amount of RNA, 10 µg, will be annealed with 2 µl of 50 µM of oligo dT primers and DEPC treated-water in a final volume of 18 µl. The annealing reaction will be carried out at 70°C for 3 minutes and kept in 4°C until the next extension reactions.

The extension reaction will be carried out by adding annealing mixture to the extension mixture. The extension reaction contains 5 mM MgCl₂, 1XPCR buffer II (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 125 µM of dATP, dGTP, dCTP, dTTP, 40 unit of RNase inhibitors, 5 mM DTT, and 100 units of MuLV Reverse transcriptase and annealing mixture in a final volume of 40 µl. The extension reaction will be held in 42°C for 90 minutes and followed by 70°C for 10 minutes. The cDNA pool will be stored at -70°C for the next PCR reactions.

Quantitative PCR:

PCR reactions will be carried out by adding 10 µl of diluted cDNA into 40 µl of PCR mixture. The PCR mixture contains 1X PCR buffer II (50 mM KCl, 10 mM Tris-HCl, pH 8.3 and 0.001% (w/v) gelatin), 5.5 mM MgCl₂, 0.2 mM dNTP mixture (Roche, CA), 900 nM of each DNMTs primer or 720 nM of each glyceraldehyde phosphate-3-dehydrogenase (GAPDH) primer, 154 nM of each fluorescent probes, 2.5 unit of

AmpliTaq Gold DNA polymerase. PCR reactions will be performed on a DNA Thermal Cycler, iCycler (Biorad, CA). DNA was denatured at 94°C for 10 minutes and followed by 45 cycles at 94°C for 15 seconds, 60°C for 1 minute.

The intact fluorescent probes contain one reporter and one quencher dye. The quencher at room temperature suppresses the reporter dye. During PCR cycles, Taq polymerase digests quencher dye away from the probe and results in increased reporter fluorescence. The fluorescence intensity is related to the amount of target DNA. Threshold cycle, Ct, is assigned when the fluorescent intensity exceeds 10 times the standard deviation of the baseline fluorescence. Final quantitation will be done using a comparative Ct method and expressed as n-fold difference over the housekeeping gene (GAPDH).

Tulane University Health Sciences Center
Institutional Review Board
Committee on Use of Human Subjects

Progress Report for Continuing Review of Research

Title of Study
Identification of DNA Methylation-Related Genes in
Human Leiomyomata Uteri (R0088)

Name and Mailing Address of Principal Investigator
John McLachlan, Ph.D.
Bioenvironmental Research
SL 3

Phone 985-6910 Fax 985-6428 Other E-Mail jmcclach@tulane.edu

Name of Co-Investigators Tung-Chin Chiang

Name of Funding Source (Sponsor) Greater New Orleans Foundation, Office of Naval Research

☐ A. Renew - continuing enrollment of new subjects
Submit 10 copies of this form, 10 copies of updated consent, 2 copies of updated project summary and 2 copies of most recent DSMB report.

☒ B. Renew - enrollment closed; however
☐ subjects are still receiving study treatment/intervention
☐ subjects have completed study treatment/intervention but continue in follow-up observation
☒ subject involvement completed but renewal is requested for data analysis
Submit 1 copy of this form and 1 copy of the most recent DSMB report.

☐ C. Terminate because
☐ research completed ☐ lack of funding ☐ other reason (specify)
Submit 1 copy of this form.

ANSWER QUESTIONS 1 - 6 BASED ON INFORMATION SINCE LAST REVIEW
Attach a succinct memo explaining any "yes" answers.

	YES	NO
Have any subjects withdrawn from the study either voluntarily or otherwise?	☐	☒
Have there been unanticipated problems or serious adverse events <u>at this site</u> involving risk to subjects since the last renewal which have not been previously reported to the IRB?	☐	☒
Has the risk/benefit ratio changed unfavorably as a result of any new information?	☐	☒
Is this a multi-center study with a Data Safety Monitoring Board? If yes, provide a report from the board.	☐	☒
Have there been any amendments since the last review not previously submitted to the IRB?	☐	☒
Have there been changes in subject population, recruitment, study procedures or consent procedures that <u>were not</u> submitted as amendments?	☐	☒
Are you requesting any changes in subject population, recruitment, study procedures or consent procedures as part of this renewal?	☒	☐
Are there any protocol deviations not previously reported or covered otherwise on this form?	☐	☒

ENROLLMENT	TMC	VA	MCLNO	Remote Sites
1. Total projected enrollment				
2. Total number of subjects enrolled to date				
3. Number of subjects enrolled since last renewal				

I certify that each of the above named co-investigators has accepted his/her role in this study. I agree to a continuing exchange of information with the Committee on Use of Human Subjects (IRB). I agree to obtain IRB approval before making any changes to the project. I agree to report promptly to the IRB all anticipated problems or serious adverse events involving risk to human subjects.

Signature of Principal Investigator
Signature of Primary Reviewer
Approved / Disapproved / Provisional

RECEIVED
EXPEDITED REVIEW
OCT 03 2001
TULANE MEDICAL CENTER
COMMITTEE ON USE
OF HUMAN SUBJECTS

Date 10/25/01
Meeting Date



Health Care
Services
Division

MEMORANDUM

TO: Kim Brown and Bret Funk
Tulane
Department of Biomedical Research

FROM: John Hunt, MD *John Hunt*
Chairperson, Research Review Committee

DATE: September 21, 2000

RE: Medical Record Chart Review

As requested in your letter dated September 21, 2000 approval is granted for you, Kim Brown and Bret Funk at 663-5164 to review medical records of patients for the following retrospective studies:

STUDY: Hysterectomies performed as a result of uterine fibroids.

However, the appropriate guidelines must be adhered to:

1. There is to be no patient contact. This approval is for retrospective medical record review ONLY.
2. Patients' names and/or hospital numbers are not to be used in reports or Publications in order to protect the right of confidentiality.
3. Permission of the respective LSU or Tulane medical school department chairpersons must be obtained if patient participants are attended by physicians other than from the researcher's school.

The Medical Records Department will pull all records that are requested for non-patient care activity within 24 hours. Inactive medical records and all death records are filed at an off-site warehouse and will be retrieved by the Medical Records' staff on a timely basis, subject to staffing schedules.

To contact Medical Records re this request, call 568-3661.

cc: Medical Records Department



Tulane University Medical Center

Office of the
Senior Vice President
for the Health Sciences
1430 Tulane Avenue
New Orleans, Louisiana 70112-2699

Committee on Use of Human Subjects TW36
Office of the Dean
Tidewater Bldg. Room 830
Phone: (504) 584-2665
Fax: (504) 586-3892

August 15, 2000

Committee Meeting
August 10, 2000

John McLachlan, Ph.D.
Center for Bioenvironmental Research
Box, SL 3

Dear Dr. McLachlan:

The Committee on Use of Human Subjects reviewed and approved the request to re-open the previously approved study entitled **Identification of DNA Methylation-Related Genes in Human Leiomyomata Uteri (R0088)** and to change the principal investigator to John McLachlan, M.D. dated 7/23/00, the revised protocol with a chart abstraction instrument and follow-up survey, and the revised consent forms submitted 7/31/00.

The Committee noted that changes consisted of administrative and procedural revisions and the necessary changes have been made in the revised consent forms. The Committee felt that the changes have essentially no impact on the risk/benefit ratio to the subjects.

Sincerely,

Ina Friedman MSN, NP-C

Ina Friedman, MSN, NP-C

Chair

Committee on Use of Human Subjects

IF:pb

GENERAL ASSURANCE NUMBER M1260

RECEIVED

JUL 31 2000

Tulane University Medical Center

CONSENT FORM

TULANE MEDICAL CENTER
COMMITTEE ON USE
OF HUMAN SUBJECTS

1. STUDY TITLE: Identification of DNA Methylation Related Genes in Human Leiomyomata Uteri (Uterine Fibroids)
2. STUDY SITES: Tulane University Medical Center; Medical Center of Louisiana, New Orleans; The Women's Laser Institute; Metairie
3. INVESTIGATORS: John McLachlan, PhD; Valerie Wilson, PhD; Shaunfang Li, PhD; Myron Moorehead, MD (consultant); Gloria Richard-Davis, MD (consultant); Craig J. Conard, MPH (585-6947)
4. PURPOSE: This is a five-year research study that will examine the role of genetics and the female hormone estrogen in the growth of uterine fibroids. Eligible research subjects agree to provide background information during patient interview and specimens of uterine tissue following hysterectomy/myomectomy for further study in a research laboratory.
5. SUBJECT INCLUSION CRITERIA: Patients eligible for this study include any women undergoing hysterectomy at the Medical Center of Louisiana in New Orleans or Tulane University Medical Center or myomectomy/hysterectomy at the Women's Laser Institute in Metairie with a diagnosis of uterine fibroids either before or during the surgical procedure.
6. SUBJECT EXCLUSION CRITERIA: Patients undergoing hysterectomy or myomectomy for reasons other than uterine fibroids, or patients that are later found to have a diagnosis of leiomyosarcoma (cancer) will be excluded from the study.
7. DESCRIPTION OF STUDY: Information including patient number, age, race, date of birth, address, height/weight, age at menarche, date of last normal menstrual period, menstrual history, menstrual symptoms, pregnancy history, type of history, location of fibroids, and size of uterus will be taken before surgery through interview or from the patient's chart. The findings of the operation and the final report of the physician examining the removed uterus will be obtained after the surgery.
Once the fibroid uterus has been removed from the patient, it will immediately be taken to a laboratory where several pieces of tissue, each the size of a sugar cube, will be removed, placed on ice, and taken to a research laboratory for further study. The total number of people that will be entered in the study is approximately seven hundred fifty.
8. BENEFITS TO SUBJECTS: Enrolled subjects will receive no direct benefit from the research study, but in learning more about the causes of uterine fibroids, we

Revised: _____ (Prior to 7/31/00)
Approved: 7/13/98
Signed before: 7/13/01

_____ Initial

Putting
Footer
on
pages

Identification of DNA Methylation-Related Genes In Human Leiomyomata Uteri

Summary:

The overall goals of this project are to establish a tissue bank for uterine fibroid and normal myometrial cells. The specimens obtained will be studied to understand the basic mechanisms of fibroid growth. The preliminary studies will involve comparing DNA methylation pattern in uterine fibroid tissue and adjacent normal myometrial tissue for identification of methylation-related genes. These genes and their significance in the growth and development of uterine fibroids will be further explored. Additionally, race-related expression of these genes will be evaluated. A clinical component will involve a chart review of patients having a hysterectomy and a follow-up component of women who had a myomectomy to determine fibroid recurrence.

I. Specific Aims

Specific Aim I:

To compare the DNA methylation pattern found in human uterine leiomyomatous tissue and adjacent normal myometrial tissue for the purpose of identifying methylation-related genes and determining their significance in the growth and development of uterine leiomyomata.

Specific Aim II:

To examine the race-related expression of several genes in human uterine leiomyomata, noting any differences; special attention will be given to the expression of a newly-cloned cytochrome cyp11b gene in our patient population.

Specific Aim III:

To characterize the study population. . .

Specific Aim IV:

Follow-up patients who have had myomectomy to determine fibroid recurrence

II. Background and Significance

Despite their classification as benign gynecological disease, leiomyomata uteri—commonly referred to as uterine fibroids or fibroid tumors—account for substantial morbidity among women of reproductive age and pose a significant public health concern. Leiomyomata are the single most common neoplasms of the female genital tract, afflicting between 20% to 50% of women based upon clinical and postmortem observations. ¹ Importantly, these tumors exhibit a higher incidence in African-Americans, and may affect black women up to nine times more frequently than their white counterparts. ² Recent reports also continue to support a disproportionate incidence of uterine fibroids, hysterectomy, and high morbidity and mortality rates among African-American. ¹⁰ Furthermore, because symptomatic fibroids often fail medical management, they represent the number one indication for hysterectomy in the United States and are involved in about 67% of these cases overall.³ National Center for Health statistics from 1965 to 1984 reflect that 27% of hysterectomies performed in the United States during that time period were specifically for symptomatic uterine fibroids. ¹¹ To date, however, little is known of the precise mechanism of development for these tumors.

Multiple clinical analyses have attempted to determine patient risk factors for the development of uterine leiomyomata. Established positive demographic factors for this disease include early menarche, premenopausal status, nulliparity, high body mass index,

and higher education, while negative factors include postmenopausal status, multiparity, and cigarette use; ⁴ the relationship of oral contraceptive use, if any, remains controversial. ⁵ Collective consideration of these factors strongly suggests an etiological role for estrogen utilization provides further support of this theory.⁶

Estrogen receptor status has been extensively studied in leiomyomatous and normal myometrial tissue. Although available data generally indicates that the expression of these receptors is greater in fibroid tissue, ⁷ the pathophysiological relevance of this observation has yet to be determined.⁸ Moreover, since estrogen is known to upregulate the induction of progesterone receptors, the effects of progesterone and the changes in the relative ratios of both hormone receptors in normal and affected tissue also merit further investigation.⁷ Nonetheless, since estrogen has been demonstrated to promote the expression of genes for insulin-like growth factor (IGF) I—a cytokine shown to enhance growth of uterine leiomyomata in vitro—it appears likely that estrogen exerts some significant effect upon tumor growth at the molecular level.^{7,9}

Growing evidence suggest that environmental exposures to a variety of compounds plays a significant role in the etiology and/or exacerbation of many diseases in women. Estrogen has been associated as a causal agent in diseases of the uterus and breast, including cancer. Environmental chemicals in water can even function as estrogen-mimicking compounds. The White House and government agencies have named environmental estrogens as a priority concern for human and environmental health. To address this concern, the CBR has embarked on a research program focusing on the environment and women's health. Specifically, the project hopes to define the role of environmental chemicals in water that has estrogen-like functions in the development and progression of uterine fibroids.

The cytochrome P450 enzymes are a multi-gene superfamily of constitutive and inducible enzymes which play a central role in the oxidative metabolism of a diverse range of xenobiotics, including carcinogens, therapeutic drugs, and several groups of biologically active endogenous compounds, such as steroid hormones and fatty acids. Recently, a new member of the cypl gene family, cyplbl, has been characterized. Estrogen-induced expression of cyplbl has been shown to occur in studies of mouse uteri, and the expectation exists that this enzyme will exhibit altered expression in leiomyomatous tissue. Examination of cyplbl expression in human leiomyomatous tissue samples will assist in further delineating the relationship of endogenous and environmental estrogens to fibroid tumor development.

Additionally, DNA methylation patterns are known to have an effect upon both normal and abnormal cell growth and differentiation. For example, the adenomatous polyposis coli (Apc) gene that causes intestinal neoplasia is suppressed by DNA hypomethylation, and a CpG island in the estrogen receptor gene of human breast cancer cells exhibits hypermethylation. It is certainly plausible that DNA methylation may play a similar role in the formation and growth of uterine leiomyomata. Through the utilization of a new technique called methylation-sensitive restriction fingerprinting (MSRF), we will screen for methylation-related genes in human fibroid development.

III. Study Subject

Patients eligible for enrollment in the study include any women undergoing hysterectomy at the Medical Center of Louisiana in New Orleans, or Tulane University Medical Center or myomectomy/hysterectomy at Lakeside Hospital (Metairie) with diagnosed uterine leiomyomata either prior to or during the surgical procedure.

IV. Protection of Participants

The results of the study will be released to the funding agency and may be published. Any information obtained from the tissue samples will be treated as confidential and referred to in publications in such a manner that the identity of all patients enrolled shall remain anonymous.

V. Materials and Methods

Clinical and Demographic Data:

Information including patient number, age, race, date of birth, address, height/weight, age at menarche, date of last normal menstrual period, menstrual history, menstrual symptoms, pregnancy history, type of history, location of fibroids, and size of uterus will be obtained either from the patient's chart or through patient interview preoperatively; documented operation findings and pathology reports will be obtained postoperatively. The total sample size is approximately 750.

The follow-up component will request information including symptoms following surgery, fibroid recurrence, treatment for fibroid recurrence, and fertility status. This information will be assessed via mail survey to patients randomly selected from a database of women who previously underwent a myomectomy. The total sample size is approximately 300.

Tissue Samples:

Fibroids or uteri and fibroid tissue removed during surgery will be immediately transported to the pathology laboratory where multiple tissue specimens measuring approximately one cubic centimeter will be obtained. These will include at least one sample of leiomyomatous tissue and one sample of grossly normal endometrial tissue. Once removed, the specimens will be placed on ice and transported to the Center for Bioenvironmental Research laboratory where they will be stored at -70°C until they can be processed.

DNA Extraction:

High molecular weight DNA will be extracted using the proteinase K method: One gram of frozen tissue is ground with a pre-chilled mortar and pestle or crushed with a hammer into a fine powder. The tissue is suspended in 10 ml of digestion buffer in tightly-capped test tubes and then incubated at 50°C in a shaking device for 12 to 18 hours. The

samples are then thoroughly extracted using an equal volume of phenol/chloroform/isoamyl alcohol. Centrifugation of the specimens is next done at 2000 rpm in a swinging bucket rotor. The aqueous (top) layer is transferred to new tube and 1/10 volume of 5M NaCl and 2 (original) volume of 100% ethanol are added. DNA is recovered by centrifugation at 2000 rpm for 2 minutes, followed by pellet rinsing with 70% ethanol. The ethanol is decanted and the pellet is air dried. DNA is resuspended in TE buffer until dissolved and stored at 4°C.

RNA Extraction:

RNA will be extracted using the guanidium isothiocyanate (GIT)/cesium chloride (CsCl₂) method: One gram of frozen tissue is ground with 20ml of guanidium in a tissuemizer with two or three 10 second bursts for complete grinding. The supernatant is collected and 0.1 volume of 20% sarkosyl is added; the mixture is heated for 2 minutes at 65°C. 0.1 gram of CsCl in an SW-28 rotor at 25,000 rpm and 25°C. The supernatant is carefully removed from the tube, the tube is inverted and drained, and the bottom of tube is cut off to obtain the RNA pellet. The pellet is placed in a 50 ml plastic tube and 3 ml of tissue resuspension buffer is added. The pellet is allowed to resuspend overnight at 4°C and then the solution is extracted sequentially using 25:24:1 phenol/chloroform/isoamyl alcohol and 24:1 chloroform/isoamyl alcohol. 0.1 volume of 3M sodium acetate (pH 5.2) and 2.5 volume of 100% ethanol are added. The mixture is then precipitated, the RNA is resuspended in water, and the RNA is quantified and stored at -80°C for use.

Methylation-Sensitive Restriction Fingerprinting:

Genomic DNA will be digested with Mse I alone or digested with BstU I and Mse I at 10 units per µg DNA following the conditions recommended by the supplier (New England Biolabs). The PCR reaction will be performed with the digested DNA (20-100ng) in 25 µl volume containing a pair of short arbitrary primers and 2 µCi ³²P dCTP. The products will be loaded onto the 4.5% polyacrylamide gel. After electrophoresis, wet gels will be dried and subjected to autoradiography at -70°C for 24 to 48 hours.

Cloning and Sequencing:

Target bands will be excised from polyacrylamide gels and gel fragments will be extracted. DNA will be eluted in water and reamplified by 30 cycles of PCR with appropriate primers. The amplified DNAs will be purified using PCR prep kit and cloned into M13mp19 vectors for sequencing.

RT-PCR: RT-PCR will be taken using the standard method to amplify the cyplbl gene from the RNAs.

Genomic Southern Hybridization:

Genomic DNAs (10µg) will be digested with appropriate restriction enzymes. These DNA digests will be resolved on 1.5% agarose gel, transferred onto a HybondTM N nylon filter and hybridized with the ³²P-labeled probes according to the gene sequence using QuiK-HyB Hybridization solution.

VI. References

1. VeKaut BS. Changing trends in the treatment of leiomyomata uteri. Current Opinion in Obstetrics and Gynecology. 5:301, 1993.
2. Witherspoon JT, Butler VW. The etiology of uterine fibroids with special reference to the frequency of occurrence in the Negro: a hypothesis. Surgical Gynecology and Obstetrics. 58:57 – 61, 1986.
3. Chryssikopoulos A, Loghis C. Indications and results of total hysterectomy. International Surgery. 71:188, 1986.
4. Samadi Ar, Lee NC, Flanders WD, et al. Risk factors for self-reported uterine fibroids: a case-control study. American Journal of Public Health. 86(6): 858 – 862m 1996,
5. Parrazini F, Vecchia CL, Negri E, et al. Oral contraceptive use and risk of uterine Fibroids. Obstetrics and Gynecology 79:430 – 3, 1992.
6. Thompson JD, Rock JA. Te Linde's Operative Gynecology, 8th Edition. Philadelphia, Lippencott-Raven Publishers, 1997, pg. 731-770.
7. Tiltman AJ. Smooth muscle neoplasms of the uterus. Current Opinion in Obstetrics and Gynecology. 9:48 – 51, 1997.
8. Poltgeiter HC, Magagone F, Bester MJ. Oestrogen and progesterone receptor and PgR/ER ratios in normal and myomatous human myometrium. East African Medical Journal. 72(8): 510 – 514, 1995
9. Strawn EY, Novy MF, Burry KA, et al. Insulin-like growth factor I promotes leiomyoma cell growth in vitro. American Journal of Obstetrics and Gynecology. 172: 1837, 1995
10. Kjerulff H, Guzinski GM, Langenberg PW, et al. Hysterectomy and Race. Obstetrics and Gynecology. 82:757 – 764, 1993
11. Pokras R, Hufnagel VG. Hysterectomy in the United States, 1965-1984. National Center for Health Statistics, Vital and Health Statistics. Series 13, Number 92, Washington DC: Government Printing Office, 1987 (DHHS publication no. (PHS) 88-1753).



4720 L-10 Service Road #100, Metairie, Louisiana 70001

FAX COVER SHEET

DATE: 6/23/99 TIME: _____
TO: Dr. Val Seltman PHONE: 585-6428
FAX: _____
FROM: Gloria Richard Smith PHONE: 804 / 454-2169
FAX: 540 / 888-3250
RE: _____
CC: _____

NUMBER OF PAGES INCLUDING COVER PAGE: 3

MESSAGE:

- ① I.R.B. Renewal
Please, complete questions 2, 4-10 on form
& return to address on form.
Send me copy of completed form.
- ② Abstract of article we discussed



4720 L-10 Service Road #100, Metairie, Louisiana 70001

FAX COVER SHEET

FILE: R0088

DATE FORWARDED: 6/04/99

FIRST REQUEST
RETURN BY: 06/21/99

Tulane University Medical Center
COMMITTEE ON USE OF HUMAN SUBJECTS
OFFICE OF THE DEAN - TIDEWATER BLDG. ROOM 830
Mail: TW-36

Tulane Medical Center must comply with FEDERAL REGULATIONS regarding initial and continuing review of all grants, contracts or studies involving human subjects.

The approval for the use of human subjects will expire this month on this study. COMPLETE, SIGN AND DATE this form and return to this Office for Committee Review by the above date. Failure to receive IRB re-approval by the expiration date will result in suspension of any further accrual/recruitment of subjects or in closure of the study.

Principal Investigator: Gloria Richard-Davis M.D.
Department: OB/GYN
Section:

MAIL BOX: SL-11

1. Title Identification of DNA Methylation-Related Genes in Human Leiomyomata Uteri (R0088)

Date originally approved: 07/13/98

Last Review: 00/00/00

2. Date initiated:

3. (Please check one)

Date completed:

or: On-going

Pending:

Cancel:

4. Where performed:

5. Number of subjects to date:

6. Informed consent obtained for each subject? Yes No

7. Records of subjects available? Yes No

8. Any deviation from original not previously reported: Yes No

9. Any problems which should be discussed with Committee? Yes No

10. Any significant adverse reactions observed not previously reported: Yes No

Gloria Richard-Davis M.D.

Investigator

6/23/99

Date

Primary Reviewer

Meeting Date

Approved / Disapproved / Provisional

Revised: 10/95

TULANE UNIVERSITY MEDICAL CENTER

CONSENT FORM

1. **STUDY TITLE:** Identification of DNA Methylation-Related Genes in Human Leiomyomate Uteria (Uterine Fibroids)
2. **STUDY SITES:** Tulane University Medical Center, Medical Center of Louisiana, New Orleans
3. **INVESTIGATORS:** Gloria Richard-Davis, M.D. (584-3774), Rachele M. Williams, pager (843-5680)
4. **PURPOSE:** This is a five-year research study that will examine the role of genetics and the female hormone estrogen in the growth of uterine fibroid tumors. Eligible research subjects agree to provide background information during patient interview and specimens of uterine tissue following hysterectomy for further study in a research laboratory.
5. **SUBJECT INCLUSION CRITERIA:** Patients eligible for this study include any women undergoing hysterectomy at the Medical Center of Louisiana in New Orleans or Tulane University Medical Center with a diagnosis of uterine fibroids either before or during the surgical procedure.
6. **SUBJECT EXCLUSION CRITERIA:** Patients undergoing hysterectomy for reasons other than uterine fibroids, or patients that are later found to have a diagnosis of leiomyosarcoma (cancer) will be excluded from the study.
7. **DESCRIPTION OF STUDY:** Information including patient name, patient number, age, race, number of children, cigarette use, symptoms, date of last normal menstrual period, menstrual history, diagnosis before surgery, and past or present hormone or birth control pill use will be taken before surgery through interview or from the patient's chart. The findings of the operation and the final report of the physician examining the removed uterus will be obtained after the surgery.

Once the fibroid uterus has been removed from the patient, it will immediately be taken to a laboratory where several pieces of tissue, each about the size of a sugar cube, will be removed, placed on ice, and taken to a research laboratory for further study. The total number of people that will be entered into the study is approximately five hundred.
8. **BENEFITS TO SUBJECTS:** Enrolled subjects will receive no direct benefit from the research study, but in learning more about the causes of uterine fibroids, we ultimately hope to decrease the medical costs and suffering for future patients with this illness.

_____ Initial

9. RISKS TO SUBJECTS: There are no risks to the study subjects in this study.
10. ALTERNATIVES TO PARTICIPATION IN THE STUDY: N/A
11. SUBJECT REMOVAL: Subjects will be removed from the study if they are found to have either of the exclusion criteria detailed under item 6 above.
12. SUBJECT'S RIGHT TO REFUSE TO PARTICIPATE OR WITHDRAW: Study subjects may refuse to participate or withdraw from the study without jeopardizing, in any way, their medical treatment at this institution in the present or future. Should significant new findings develop during the course of the research which may relate to the subject's willingness to continue participation, that information will be provided to the subject.
13. SUBJECT'S RIGHT TO PRIVACY: The results of the study may be released to the funding agency. The results of the study may be published. The privacy of subjects will be protected and their names will not be used in any manner.
14. FINANCIAL INFORMATION: Participation in this study will not result in any extra charges above and beyond those routinely incurred by patients with similar illnesses. I understand that I will not receive financial remuneration for participating in this study. I understand that Tulane University Medical Center and the investigators in this protocol will provide necessary medical treatment for any injury or illness which may arise from my participation in this research protocol. However, such medical treatment will be on a fee-for-service basis, payable by myself or my insurance carrier if covered by them. I understand that the Administrators of the Tulane Educational Fund, doing business as Tulane University Medical Center do not provide any form of compensation for injuries or illness arising from participation in this research protocol, including but not limited to free medical care other than as described in this consent form, if any, payment of lost wages, or compensation for pain or suffering. I understand that complete information concerning the non-availability of compensation and the availability of treatment can be obtained from the Office of the Assistant General Counsel, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, Louisiana 70112, (504) 588-5031. Additionally, that office is available to answer questions about subject's rights.

_____ Initial

I have read and understand that this information stated above and I sign this consent form willingly. I have received a copy of this consent form.

Patient

Date

Parent/Guardian

Date

Witness

Date

I am unable to read but this consent has been read and explained to me by _____
(name of reader). I understand the information stated above and I willingly sign this consent form.

Patient

Date

Parent/Guardian

Date

Witness

Date

Initial



Tulane University Medical Center

Office of the Chancellor
1430 Tulane Avenue
New Orleans, Louisiana 70112-2699

Committee on Use of Human Subjects TW36
Office of the Dean
Tidewater Bldg. Room 830
Phone: (504) 584-2665
Fax: (504) 586 3892

Val's copy

TO INVESTIGATOR:

The Committee on Use of Human Subjects reviewed and approved the study listed in the enclosed letter of certification.

It is your responsibility to forward the enclosed certification of the Committee's review and approval to the granting agency.

If your study involves patients or facilities at Tulane Medical Center, La. Health Care Authority/Medical Center of Louisiana in New Orleans, Veterans Administration Medical Center of New Orleans or G.W.L. Hansen's Disease Center in Baton Rouge, it is your responsibility to inform the Medical Director of the institution(s) prior to initiating your research project. You are reminded that these are the only institutions where you may enroll patients. The Committee presumes, unless otherwise stated, that minors and mentally incompetent individuals are not to be enrolled in this study.

Sincerely,

William Robinson

William R. Robinson, III, M.D.
Chairman
Committee on Use of Human Subjects

Enclosure

David M. Mushatt, M.D.
Vice Chairman

John Beal, J.D.
Alternate Vice Chairman

GENERAL ASSURANCE NUMBER M1260



Tulane University Medical Center

Office of the Chancellor
1430 Tulane Avenue
New Orleans, Louisiana 70112-2699

Committee on Use of Human Subjects TW36
Office of the Dean
Tidewater Bldg. Room 830
Phone: (504) 584-2665
Fax: (504) 586-3892

DATE: July 13, 1998

This is to certify that the grant, contract or study with the consent form dated 6/29/98 entitled

Identification of DNA Methylation-Related Genes in Human Leiomyomata Uteri (R0088)

submitted by:

NAME: Gloria Richard-Davis, M.D.
TITLE: Principal Investigator

for consideration has been reviewed by the Committee and approved with respect to the study of human subjects as adequately protecting the rights and welfare of the individuals involved, employing adequate methods of securing legally effective informed consent from these individuals and not involving undue risk in the light of the potential medical benefits to be derived therefrom.

This IRB is in compliance with the requirements in Part 56, Subchapter D, Part 312 of the 21 Code of Federal Regulations, published January 27, 1981.

HUMAN SUBJECTS - REVIEWED AT RISK

MEETING: June 11, 1998

A handwritten signature in cursive script, reading "William R. Robinson, III".

APPROVED ON: July 13, 1998

William R. Robinson, III, M.D.
Chairman
Committee on Use of Human Subjects

GENERAL ASSURANCE NUMBER M1260



Tulane University Medical Center

Office of the Chancellor
1430 Tulane Avenue
New Orleans, Louisiana 70112-2699

Committee on Use of Human Subjects TW36
Office of the Dean
Tidewater Bldg. Room 830
Phone: (504) 584-2665
Fax: (504) 586-3892

June 12, 1998
COMMITTEE MEETING
June 11, 1998

Gloria Richard-Davis, M.D.
Department of OB/GYN
Box SL 11

Dear Dr. Richard-Davis:

The Committee on Use of Human Subjects reviewed and approved the study entitled **Identification of DNA Methylation-Related Genes in Human Leiomyomata Uteri (R0088)** upon acceptance of the following provisions:

- 1) The wallet card statement needs to be deleted from the consent form.
- 2) The consent form needs to have a place at the bottom of each page of the consent form for the patient's initials.

Final approval will be granted upon submission of your response in writing to the I.R.B. Office in care of Erna Bauer. Please hi-light all additional or revised information. No subjects may be enrolled until final approval is granted.

Sincerely,

William R. Robinson, III, M.D.
Chairman
Committee on Use of Human Subjects

WRR:pb

GENERAL ASSURANCE NUMBER M1260

TULANE UNIVERSITY MEDICAL CENTER

CONSENT FORM

1. **STUDY TITLE:** Identification of DNA Methylation-Related Genes in Human Leiomyomata Uteri (Uterine Fibroids)
2. **STUDY SITES:** Tulane University Medical Center, Medical Center of Louisiana New Orleans
3. **INVESTIGATORS:** Gloria Richard-Davis, M.D. (584-3774), Kenneth K. Moghadam (896-9927)
4. **PURPOSE:** This is a five-year research study that will examine the role of genetics and the female hormone estrogen in the growth of uterine fibroid tumors. Eligible research subjects agree to provide background information during patient interview and specimens of uterine tissue following hysterectomy for further study in a research laboratory.
5. **SUBJECT INCLUSION CRITERIA:** Patients eligible for this study include any women undergoing hysterectomy at the Medical Center of Louisiana in New Orleans or Tulane University Medical Center with a diagnosis of uterine fibroids either before or during the surgical procedure.
6. **SUBJECT EXCLUSION CRITERIA:** Patients undergoing hysterectomy for reasons other than uterine fibroids, or patients that are later found to have a diagnosis of leiomyosarcoma (cancer) will be excluded from the study.
7. **DESCRIPTION OF STUDY:** Information including patient name, patient number, age, race, number of children, cigarette use, symptoms, date of last normal menstrual period, menstrual history, diagnosis before surgery, and past or present hormone or birth control pill use will be taken before surgery through interview or from the patient's chart. The findings of the operation and the final report of the physician examining the removed uterus will be obtained after the surgery.

Once the fibroid uterus has been removed from the patient, it will immediately be taken to a laboratory where several pieces of tissue, each about the size of a sugar cube, will be removed, placed on ice, and taken to a research laboratory for further study. The total number of people that will be entered in the study is approximately five hundred.
8. **BENEFITS TO SUBJECTS:** Enrolled subjects will receive no direct benefit from the research study, but in learning more about the causes of uterine fibroids, we ultimately hope to decrease the medical costs and suffering for future patients with this illness.
9. **RISKS TO SUBJECTS:** There are no risks to the study subjects in this study.
10. **ALTERNATIVES TO PARTICIPATION IN THE STUDY:** N/A
11. **SUBJECT REMOVAL:** Subjects will be removed from the study if they are found to have either of the exclusion criteria detailed under item 6 above.
12. **SUBJECTS RIGHT TO REFUSE TO PARTICIPATE OR WITHDRAW:** Study subjects may refuse to participate or withdraw from the study without jeopardizing, in any way, their medical treatment at this institution in the present or future. Should significant new findings develop during the course of the research which may relate to the subject's willingness to continue participation, that information will be provided to the subject.

13. **SUBJECT'S RIGHT TO PRIVACY:** The results of the study may be released to the funding agency. The results of the study may be published. The privacy of subjects will be protected and their names will not be used in any manner.
14. **RELEASE OF INFORMATION:** The medical records of the study are available to both the Center for Bioenvironmental Research and the Food and Drug Administration.
15. **FINANCIAL INFORMATION:** Participation in this study will not result in any extra charges above and beyond those routinely incurred by patients with similar illnesses. I understand that I will not receive financial remuneration for participating in this study.
I understand that Tulane University Medical Center and the investigators in this protocol will provide necessary medical treatment for any injury or illness which may arise from my participation in this research protocol. However, such medical treatment will be on a fee-for-service basis, payable by myself or my insurance carrier if covered by them.
I understand that the Administrators of the Tulane Educational Fund, doing business as Tulane University Medical Center do not provide any form of compensation for injuries or illness arising from participation in this research protocol, including but not limited to free medical care other than as described in this consent form, if any, payment of lost wages, or compensation for pain or suffering.
I understand that complete information concerning the non-availability of compensation and the availability of treatment can be obtained from the Office of the Assistant General Counsel, Tulane University Medical School, 1430 Tulane Avenue, New Orleans, Louisiana 70112, (504) 588-5031. Additionally, that office is available to answer questions about subject's rights.
16. **SIGNATURES:** I understand that I will be given a wallet size card to be carried in my purse or wallet stating the name of the study I am participating in and the name and telephone number of the contact person in the event of an emergency.

I have read and understand this information stated above and I sign this consent form willingly. I have received a copy of this consent form.

Patient

Date

Parent/Guardian

Date

Witness

Date

I am unable to read but this consent has been read and explained to me by _____
(name of reader). I understand the information stated above and I willingly sign this consent form.

Patient

Date

Parent/Guardian

Date

Witness

Date

THIS CHECKLIST WILL ASSIST IN A MORE EFFICIENT AND SPEEDY PROCESS
COMMITTEE ON USE OF HUMAN SUBJECTS

II. CONSENT FORM

1. X Title of study and page number on all pages
2. X Description of procedure or drug in lay language
3. NA Risks and/or side effects
4. NA Standard blood drawing statement (if applicable)
5. X Benefits to subject direct or indirect to society
6. X Assurance of voluntary participation
7. X Assurance of right to withdraw anytime without prejudice
8. NA Disclosure of alternate treatment
9. X Assurance of disclosure of new findings
10. NA Additional costs to subject or financial remuneration
11. X Offer to answer questions with physician's name and phone number
12. X Name and phone number of contact person in case of study related emergency
13. X Assurance of confidentiality with clarification that study falls under FDA jurisdiction and therefore FDA has access to all study information
14. X Card for outpatients to carry in purse or wallet stating name of study and contact person's name and phone number in case of study related emergency
15. X Compensation statement
16. X Dean's statement
17. X Read statement with signature lines for subject and witness
18. X Parent/guardian statement with signature lines
19. NA Assent statement for minors 12 - 18 years of age, unmarried or otherwise unemancipated with signature lines for minor and witness/reader

**Identification of DNA Methylation-Related Genes
in
Human Leiomyomata Uteri**

Summary:

The overall goals of this project is to establish a tissue bank for uterine fibroid and normal myometrial cells. The specimens obtained will be studied to understand the basic mechanisms of fibroid growth. The preliminary studies will involve comparing DNA methylation pattern in uterine fibroid tissue and adjacent normal myometrial tissue for identification of methylation-related genes. These genes and their significance in the growth and development of uterine fibroids will be further explored. Additionally, race-related expression of these genes will be evaluated.

Identification of DNA Methylation-Related Genes in
Human Leiomyomata Uteri

I. Specific Aims

Specific Aim I:

To compare the DNA methylation pattern found in human uterine leiomyomatous tissue and adjacent normal myometrial tissue for the purpose of identifying methylation-related genes and determining their significance in the growth and development of uterine leiomyomata.

Specific Aim II:

To examine the race-related expression of several genes in human uterine leiomyomata, noting any differences; special attention will be given to the expression of a newly-cloned cytochrome cyp1b1 gene in our patient population.

II. Background and Significance

Despite their classification as benign gynecological disease, leiomyomata uteri--commonly referred to as uterine fibroids or fibroid tumors--account for substantial morbidity among women of reproductive age and pose a significant public health concern. Leiomyomata are the single most common neoplasms of the female genital tract, afflicting between 20% to 50% of women based upon clinical and postmortem observations.¹ Importantly, these tumors exhibit a higher incidence in African-Americans, and may affect black women up to nine times more frequently than their white counterparts.² Recent reports also continue to support a disproportionate incidence of uterine fibroids, hysterectomy, and high morbidity and mortality rates among African-Americans.¹⁰ Furthermore, because symptomatic fibroids often fail medical management, they represent the number one indication for hysterectomy in the United States and are involved in about 67% of these cases overall.³ National Center for Health statistics from 1965 to 1984 reflect that 27% of hysterectomies performed in the United States during that time period were specifically for symptomatic uterine fibroids.¹¹ To date, however, little is known of the precise mechanism of development for these tumors.

Multiple clinical analyses have attempted to determine patient risk factors for the development of uterine leiomyomata. Established positive demographic factors for this disease include early menarche, premenopausal status, nulliparity, high body mass index, and higher education, while negative factors include postmenopausal status, multiparity, and cigarette use;⁴ the relationship of oral contraceptive use, if any, remains controversial.⁵ Collective consideration of these factors strongly suggests an etiological role for estrogens in the pathogenesis of uterine leiomyomata, and the noted growth of fibroid tumors during pregnancy and high-dose estrogen utilization provides further support of this theory.⁶

Estrogen receptor status has been extensively studied in leiomyomatous and normal myometrial tissue. Although available data generally indicates that the expression of these receptors is greater in fibroid tissue,⁷ the pathophysiological relevance of this observation has yet to be

determined.⁸ Moreover, since estrogen is known to upregulate the induction of progesterone receptors, the effects of progesterone and the changes in the relative ratios of both hormone receptors in normal and affected tissue also merit further investigation.⁷ Nonetheless, since estrogen has been demonstrated to promote the expression of genes for insulin-like growth factor (IGF) I--a cytokine shown to enhance growth of uterine leiomyomata in vitro--it appears likely that estrogen exerts some significant effect upon tumor growth at the molecular level.^{7,9}

Growing evidence suggest that environmental exposures to a variety of compounds plays a significant role in the etiology and/or exacerbation of many diseases in women. Estrogen has been associated as a causal agent in diseases of the uterus and breast, including cancer. Environmental chemicals in water can even junction as estrogen-mimicking compounds. The White House and government agencies have named environmental estrogens as a priority concern for human and environmental health. To address this concern, the CBR has embarked on a research program focusing on the environment and women's health. Specifically, the project hopes to define the role of environmental chemicals in water that has estrogen-like functions in the development and progression of uterine fibroids.

The cytochrome P450 enzymes are a multi-gene superfamily of constitutive and inducible enzymes which play a central role in the oxidative metabolism of a diverse range of xenobiotics, including carcinogens, therapeutic drugs, and several groups of biologically active endogenous compounds, such as steroid hormones and fatty acids. Recently, a new member of the CYP1 gene family, *cyp1b1*, has been characterized. Estrogen-induced expression of *cyp1b1* has been shown to occur in studies of mouse uteri, and the expectation exists that this enzyme will exhibit altered expression in leiomyomatous tissue. Examination of *cyp1b1* expression in human leiomyomatous tissue samples will assist in further delineating the relationship of endogenous and environmental estrogens to fibroid tumor development.

Additionally, DNA methylation patterns are known to have an effect upon both normal and abnormal cell growth and differentiation. For example, the adenomatous polyposis coli (*Apc*) gene that causes intestinal neoplasia is suppressed by DNA hypomethylation, and a CpG island in the estrogen receptor gene of human breast cancer cells exhibits hypermethylation. It is certainly plausible that DNA methylation may play a similar role in the formation and growth of uterine leiomyomata. Through the utilization of a new technique called methylation-sensitive restriction fingerprinting (MSRF), we will screen for methylation-related genes in human fibroid development.

III. Study Subjects

Patients eligible for enrollment in the study include any women undergoing hysterectomy at the Medical Center of Louisiana in New Orleans or Tulane University Medical Center with diagnosed uterine leiomyomata either prior to or during the surgical procedure.

IV. Protection of Participants

The results of the study will be released to the funding agency and may be published. Any information obtained from the tissue samples will be treated as confidential and referred to in publications in such a manner that the identity of all patients enrolled shall remain anonymous.

V. Materials and Methods

Clinical and Demographic Data:

Information including patient number, age, parity, race, height, weight, smoking history, clinical symptoms, last normal menstrual period, gynecological history, preoperative diagnosis, and hormonal (oral contraception pills, estrogen, progesterone, GnRH agonist) medication use will be obtained either from the patient's chart or through patient interview preoperatively; documented operation findings and pathology reports will be obtained postoperatively.

Tissue Samples:

Fibroids or uteri and fibroid tissue removed during surgery will be immediately transported to the pathology laboratory where multiple tissue specimens measuring approximately one cubic centimeter will be obtained. These will include at least one sample of leiomyomatous tissue and one sample of grossly normal myometrial tissue. Once removed, the specimens will be placed on ice and transported to the Center for Bioenvironmental Research laboratory where they will be stored at -70°C until they can be processed.

DNA Extractions:

High molecular weight DNA will be extracted using the proteinase K method: One gram of frozen tissue is ground with a pre-chilled mortar and pestle or crushed with a hammer into a fine powder. The tissue is suspended in 10 ml of digestion buffer in tightly-capped test tubes and then incubated at 50°C in a shaking device for 12 to 18 hours. The samples are then thoroughly extracted using an equal volume of phenol/chloroform/ isoamyl alcohol. Centrifugation of the specimens is next done at 2000 rpm in a swinging bucket rotor. The aqueous (top) layer is transferred to new tube and 1/10 volume of 5M NaCl and 2 (original) volume of 100% ethanol are added. DNA is recovered by centrifugation at 2000 rpm for 2 minutes, followed by pellet rinsing with 70% ethanol. The ethanol is decanted and the pellet is air dried. DNA is resuspended in TE buffer until dissolved and stored at 4°C .

RNA Extraction:

RNA will be extracted using the guanidium isothiocyanate (GIT)/cesium chloride (CsCl_2) method: One gram of frozen tissue is ground with 20 ml of guanidium in a tissuemizer with two or three 10 second bursts for complete grinding. The tissue is then centrifuged 10 minutes in an SS-34 rotor at 10,000 rpm at 12°C . The supernatant is collected and 0.1 volume of 20% Sarkosyl is added; the mixture is heated for 2 minutes at 65°C . 0.1 gram of CsCl / ml of solution is added, dissolved, and then the sample is layered over 9 ml of 5.7M CsCl in an SW-28 silanized and autoclaved in a polyallomer tube. The specimen is centrifuged overnight in an SW-28 rotor at 25,000 rpm and 25°C . The supernatant is carefully removed from the tube, the tube is inverted

and drained, and the bottom of tube is cut off to obtain the RNA pellet. The pellet is placed in a 50 ml plastic tube and 3 ml of tissue resuspension buffer is added. The pellet is allowed to resuspend overnight at 4°C and then the solution is extracted sequentially using 25:24:1 Phenol/chloroform/isoamyl alcohol and 24:1 chloroform/isoamyl alcohol. 0.1 volume of 3M sodium acetate (pH 5.2) and 2.5 volume of 100% ethanol are added. The mixture is then precipitated, the RNA is resuspended in water, and the RNA is quantified and stored at -80°C for use.

Methylation-Sensitive Restriction Fingerprinting:

Genomic DNA will be digested with *Mse I* alone or digested with *BstU I* and *Mse I* at 10 units per µg DNA following the conditions recommended by the supplier (New England Biolabs). The PCR reaction will be performed with the digested DNA (20-100 ng) in 25 µl volume containing a pair of short arbitrary primers and 2u Ci ³²P dCTP. The products will be loaded onto the 4.5% polyacrylamide gel. After electrophoresis, wet gels will be dried and subjected to autoradiography at -70°C for 24 to 48 hours.

Cloning and Sequencing:

Target bands will be excised from polyacrylamide gels and gel fragments will be extracted. DNA will be eluted in water and reamplified by 30 cycles of PCR with appropriate primers. The amplified DNAs will be purified using PCR prep kit and cloned into M13mp19 vectors for sequencing.

RT-PCR: RT-PCR will be taken using the standard method to amplify the *cyp1b1* gene from the RNAs.

Genomic Southern Hybridization:

Genomic DNAs (10µg) will be digested with appropriate restriction enzymes. These DNA digests will be resolved on 1.5% agarose gel, transferred onto a Hybond™ N⁺ nylon filter and hybridized with the ³²P-labeled probes according to the gene sequence using Quik-Hyb Hybridization solution.

VI. References

1. VeKaut BS. Changing trends in the treatment of leiomyomata uteri. Current Opinion in Obstetrics and Gynecology. 5: 301, 1993.
2. Witherspoon JT, Butler VW. The etiology of uterine fibroids with special reference to the frequency of occurrence in the Negro: a hypothesis. Surgical Gynecology and Obstetrics. 58: 57 - 61, 1934.
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4. Samadi AR, Lee NC, Flanders WD, et al. Risk factors for self-reported uterine fibroids: a case-control study. American Journal of Public Health. 86(6): 858 - 862, 1996.

5. Parrazini F, Vecchia CL, Negri E, et al. Oral contraceptive use and risk of uterine fibroids. Obstetrics and Gynecology 79: 430 - 3, 1992.
6. Thompson JD, Rock JA. Te Linde's Operative Gynecology, 8th Edition. Philadelphia, Lippencott-Raven Publishers, 1997, pg. 731-770.
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8. Poltgeiter HC, Magagone F, Bester MJ. Oestrogen and progesterone receptor and PgR/ER ratios in normal and myomatous human myometrium. East African Medical Journal. 72(8): 510 - 514, 1995.
9. Strawn EY, Novy MF, Burry KA, et al. Insulin-like growth factor I promotes leiomyoma cell growth in vitro. American Journal of Obstetrics and Gynecology. 172: 1837, 1995
10. Kjerulff H, Guzinski GM, Langenberg PW, et al. Hysterectomy and Race. Obstetrics and Gynecology. 82: 757 - 764, 1993.
11. Pokras R, Hufnagel VG. Hysterectomy in the United States, 1965-1984. National Center for Health Statistics, Vital and Health Statistics. Series 13, Number 92, Washington DC: Government Printing Office, 1987 (DHHS publication no. (PHS) 88-1753).



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

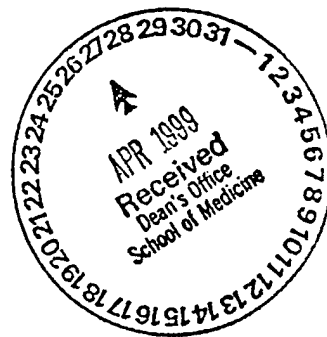
National Institutes of Health
Office for Protection from Research Risks
6100 Executive Boulevard, MSC 7507
Rockville, Maryland 20892-7507
Phone: 301-496-7041
FAX: 301-402-0527

Hand delivery or express mail:

6100 Executive Boulevard, Suite 3B01
Rockville, Maryland 20852

April 5, 1999

James J. Corrigan, Jr., MD
Dean
Tulane University Medical Center
1430 Tulane Avenue
New Orleans, LA 70112



Subject: Multiple Project Assurance M-1260

Dear Dr. Corrigan:

The Department of Health and Human Services (DHHS) has approved the renewal of your Multiple Project Assurance dated November 23, 1998 submitted by your institution in compliance with the requirements for the protection of human subjects (45 CFR 46).

Your new Assurance became effective on **March 30, 1999**, and retains the same identification number of M-1260. It will expire on **March 29, 2004**, and a new Assurance is to be negotiated with the Office for Protection from Research Risks (OPRR) prior to that date. Please reference your Multiple Project Assurance number with all future correspondence to this office.

This MPA is being approved subject to the following understandings:

- the MPA IRB membership list (Appendix C) now requires the Chair's signature. (Please see downloadable sample MPA from http://www.nih.gov:80/grants/oprr/library_human.htm); please forward a signed list at your convenience for our records.
- In addition, IRB alternates should be cross-referenced to those for whom they would substitute (either individually or by class of voting member)
- for your information and continued reference, OPRR has modified its sample Inter-Institutional Amendment (<http://www.nih.gov:80/grants/oprr/humansubjects/assurance/mpa-iaa.htm>) for posting in the immediate future to eliminate heretofore inconsistencies which are principally found at Part I, I, B, 1, a-d as follows:
 - a. the research is sponsored by the affiliated IRB institution, or

- b. the research is conducted by or under the direction of any employee or agent of the affiliated IRB institution in connection with his or her institutional responsibilities, or
- c. the research is conducted by or under the direction of any employee or agent of the affiliated IRB institution using any property or facility of the institution, or
- d. the research involves use by the affiliated IRB institution of this institution's non-public information to identify or contact human research subject or prospective subjects.

- Appendix D refers to an annual "institutional" audit of the IRB practices that's carried out by IRB personnel; as described it may be more accurate to describe this activity as IRB self-audits versus the institution reviewing adherence by the IRB to the OPRR-approved MPA.

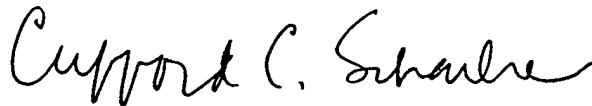
Your Institutional Review Board (IRB) has been assigned identification number 01NR. This IRB number, along with your Assurance number, will be required in certain forms (e.g., PHS-2590) and other correspondence.

The Assurance defines the relationship of your institution to DHHS since it sets out your responsibilities and the procedures that will be used by your institution to protect human subjects. Among the most important elements of the Assurance are the reporting requirements to this office and your agreement to disseminate the content of this Assurance to those individuals at your institution who are in any way associated with human subject research.

A copy of the approved Assurance is enclosed, as is a blank format sheet to facilitate the future reporting of changes in your IRB membership.

If I can be of further help, please contact me.

Sincerely,



Clifford C. Scharke, D.M.D., M.P.H.
Chief, Assurance Branch
Division of Human Subject Protections
Office for Protection from Research Risks

Enclosures



Tulane University Medical Center

Office of the Chancellor
1430 Tulane Avenue
New Orleans, Louisiana 70112-2699

Committee on Use of Human Subjects TW36
Office of the Dean
Tidewater Bldg. Room 830
Phone: (504) 584-2665
Fax: (504) 586-3892

Multiple Project Assurance of Compliance with DHHS Regulations for Protection of Human Research Subjects

Tulane Medical Center, hereinafter known as the "institution" (see Appendix A), hereby gives assurance, as specified below, that it will comply with the Department of Health and Human Services (DHHS) regulations for the protection of human research subjects, 45 CFR Part 46, as amended to include provisions of the Federal Policy for the Protection of Human Subjects (56FR28003) as Subpart A, and as may be further amended during the approval period for this Assurance.

PART 1 - PRINCIPLES, POLICIES, AND APPLICABILITY

I. Ethical Principles

- A. This institution is guided by the ethical principles regarding all research involving humans as subjects, as set forth in the report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (entitled: Ethical Principles and Guidelines for the Protection of Human Subjects of Research [the "Belmont Report"]), regardless of whether the research is subject to Federal regulation or with whom conducted or source of support (i.e., sponsorship).
- B. All institutional and non-institutional performance sites for this institution, domestic or foreign, will be obligated by this institution to conform to ethical principles which are at least equivalent to those of this institution, as cited in the previous paragraph or as may be determined by the DHHS Secretary.

II. Institutional Policy

- A. All requirements of Title 45, Part 46, of the Code of Federal Regulations (45 CFR 46) will be met for all federally-sponsored research, and all other human subject research regardless of sponsorship (1), except as otherwise noted in this Assurance. Federal (all departments and agencies bound by the Federal Policy) funds for which this Assurance applies may not be expended for research involving human subjects unless the requirements of this Assurance have been satisfied.
- B. Except for those categories specifically exempted or waived under Section 101(b)(1-6) or 101(i), all research covered by this Assurance will be reviewed and approved by an Institutional Review Board (IRB) which has been established under a Multiple Project Assurance (MPA) with OPRR or as may be otherwise agreed to by OPRR (see Part 1, II, G). The involvement of human subjects in research covered by this Assurance will not be permitted until an appropriate IRB has reviewed and approved the research protocol and informed consent has been obtained from the subject or the subject's legal representative (see Sections 111, 116, and 117), unless properly waived by the IRB under Section 116(c),(d) or by any applicable waiver under Section 101(i).
- C. This institution assures that before human subjects are involved in nonexempt research covered by this Assurance, the IRB(s) will give proper consideration to:

1. the risks to the subjects,
 2. the anticipated benefits to the subjects and others,
 3. the importance of the knowledge that may reasonably be expected to result, and
 4. the informed consent process to be employed.
- D. Certification of IRB review and approval for all Federally-sponsored research involving human subjects will be submitted to the Office of Research Administration (ORA) for forwarding to the appropriate Federal department or agency. Compliance will occur within the time and in the manner prescribed for forwarding certifications of IRB review to DHHS or other Federal departments or agencies for which this Assurance applies. As provided for under section 118, applications and proposals lacking definite plans for involvement of human subjects will not require IRB review and approval prior to award. However, except for research exempted or waived under Section 101 (b) or (i), no human subjects may be involved in any project supported by such awards until IRB review and approval has been certified to the appropriate Federal department or agency. As required under Section 119, the IRB will review proposed involvement of human subjects in Federal research activities undertaken without prior intent for such involvement, but will not permit such involvement until certification of the IRB's review and approval is received by the appropriate Federal department or agency.
- E. Institutions that are not direct signatories to this Assurance are not authorized to cite this Assurance. This institution will ensure that such other institutions and investigators not bound by the provisions of this Assurance will satisfactorily assure compliance with 45 CFR 46, as required (see Part 2, I, D and II, K), as a prior condition for involvement in DHHS-sponsored human subject research which is under the auspices of this institution (see Part 1, III, A). Institutions that have entered into an Inter-Institutional Amendment (IIA) to this Assurance must submit a Single Project Assurance (SPA) to the Office for Protection from Research Risks (OPRR) for DHHS-sponsored research, when that research is not conducted under the auspices of a signatory institution to this Assurance.
- F. This institution will ensure that any collaborating entities (i.e., those entities engaged in human subject research by virtue of subject accrual, transfer of identifiable information, and/or in exchange of something of value, such as material support [e.g., money, drugs, or identifiable specimens], coauthorship, intellectual property, or credits) materially engaged in the conduct of non-federal sponsored research involving human subjects will possess mechanisms to protect human research subjects that are at least equivalent to those procedures provided for in the ethical principles to which this institution is committed (see Part 1, I).
- G. This institution will comply with the requirements set forth in Section 114 of the regulations regarding cooperative research projects. When research covered by this Assurance is conducted at or in cooperation with another entity, all provisions of this Assurance remain in effect for that research. This institution may accept, for the purpose of meeting the IRB review requirements, the review of an IRB established under another DHHS MPA. Such acceptance must be (a) in writing, (b) approved and signed by an official of this institution's Office of Research Administration(s), and (c) approved and signed by correlative officials of each of the other cooperating institutions (i.e., a Cooperative Amendment to this MPA). The original of the signed understanding will serve as an addendum to this Assurance and will be forwarded to the OPRR of DHHS by the ORA(s) for OPRR approval.
- H. This institution will exercise appropriate administrative overview to ensure that the institution's policies and procedures designed for protecting the rights and welfare of human subjects are being effectively applied in compliance with this Assurance.
- I. Description of this institution's policy for the protection of human subjects is contained in its internal written procedures which are available to OPRR and other Federal departments or agencies, upon request. Appendix D to this Assurance abstracts pertinent organizational, personnel, and reporting procedures sufficient to describe the substance and relative prominence conferred upon the protection of subjects.

III Applicability

- A. Except for research in which the only involvement of humans is in one or more of the categories exempted or waived under Section 101(b)(1-6) or 101(i), this Assurance applies to all research involving human subjects, and all other activities which even in part involve such research, regardless of sponsorship¹, if one or more of the following apply:
1. the research is sponsored by this institution, or
 2. the research is conducted by or under the direction of any employee or agent of this institution in connection with his or her institutional responsibilities, or
 3. the research is conducted by or under the direction of any employee or agent of this institution using any property or facility of this institution, or
 4. the research involves the use of this institution's non-public information to identify or contact human research subjects or prospective subjects.
- B. All human subject research which is exempt under Section 101(b)(1-6) or 101(i) will be conducted in accordance with: (1) the Belmont Report, (2) this institution's administrative procedures to ensure valid claims of exemption, and (3) orderly accounting for such activities.
- C. Components of this institution are bound by the provisions of this Assurance. Those components which can be expected to participate in human subject research sponsored by DHHS or other Federal departments or agencies for which this Assurance applies are identified in Appendix A. Appendix A will be revised as changes occur and revisions forwarded to OPRR.
- D. This Assurance must be accepted by other Federal departments or agencies that are bound by the Federal Policy for the Protection of Human Subjects when appropriate for the research in question and therefore applies to all human subject research so sponsored.¹ Research that is neither conducted nor supported by a Federal department or agency but is subject to regulation as defined in Section 102(e) must be reviewed and approved, in compliance with Sections 101, 102, and 107 through 117.

PART 2 - RESPONSIBILITIES

I. Institution

- A. This institution acknowledges that it bears full responsibility for the performance of all research involving human subjects, covered by this Assurance, including complying with Federal, state, or local laws as they may relate to such research.
- B. This institution will require appropriate additional safeguards in research that involves: (1) fetuses, pregnant women, or human ova invitro fertilization (see 45 CFR 46 Subpart B), (2) prisoners (see 45 CFR 46 Subpart C), (3) children (see 45 CFR 46 Subpart D), (4) the cognitively impaired, or (5) other potentially vulnerable groups.
- C. This institution, including all its named components (see Appendix A), acknowledges and accepts its responsibilities for protecting the rights and welfare of human subjects of research covered by this Assurance.
- D. This institution is responsible for acquiring appropriate Assurances or Amendments, when requested, and certifications of IRB review and approval for federally sponsored research from all its standing affiliates (see Appendix B) and Assurances or Agreements for all others, domestic or foreign, which may otherwise become affiliated on a limited basis in such research.
- E. This institution is responsible for ensuring that no performance site cooperating in the conduct of federally sponsored research for which this Assurance applies does so without Federal department or agency approval of an appropriate assurance of compliance, in whatever appropriate form, and satisfaction of IRB certification requirements.
- F. In accordance with the compositional requirements of Section 107, this institution has established the IRB(s) listed in the attached roster(s) (see Appendix C). Certain research supported by the U.S. Department of Education will be reviewed in accordance with the requirements of Title 34 CFR Parts 350 and 356 which require that the IRB(s) include at least one person who is primarily concerned with the welfare of handicapped children or mentally disabled persons.
- G. This institution will provide both meeting space and sufficient staff to support the IRB's review and record-keeping duties.
- H. This institution recognizes that involvement in research activities of any OPRR-recognized Cooperative Protocol Research Programs (CPRPs) will involve additional reporting and record-keeping requirements related to human subject protections.
- I. This institution is responsible for ensuring that it and all its affiliates comply fully with all applicable Federal policies and guidelines, including those concerning notification of seropositivity, counseling, and safeguarding confidentiality where research activities directly or indirectly involve the study of human immunodeficiency virus (HIV).

II. Office of Research Administration for Human Subject Research (ORA)

- A. The ORA(s) will receive from investigators, through their supervisors, all research protocols which involve human subjects, keep investigators informed of decisions and administrative processing, and return all disapproved protocols to them.

- B. The ORA(s) is responsible for reviewing the preliminary determinations of exemption by investigators and supervisors and for making the final determination based on Section 101 of the regulations. Notice of concurrence for all exempt research will be promptly conveyed in writing to the investigator. All nonexempt research will be forwarded to the appropriate IRB.
- C. The ORA(s) will make the preliminary determination of eligibility for expedited review procedures (see Section 110). Expedited review of research activities will not be permitted where full board review is required.
- D. The ORA(s) will review all research (whether exempt or not) and decide whether the institution will permit the research. If approved by the IRB, but not permitted by the ORA, the ORA will promptly convey notice to the investigator and the IRB Chair. Neither the ORA nor any other office of the institution may approve a research activity that has been disapproved by the appropriate IRB.
- E. The ORA(s) will forward certification of IRB approval of proposed research to the appropriate Federal department or agency only after all IRB-required modifications have been incorporated to the satisfaction of the IRB.
- F. The ORA(s) will designate procedures for the retention of signed consent documents for at least three years past completion of the research activity.
- G. The ORA(s) will maintain and arrange access for inspection of IRB records as provided for in Section 115.
- H. The ORA(s) is responsible for ensuring constructive communication among the research administrators, department heads, research investigators, clinical care staff, human subjects, and institutional officials as a means of maintaining a high level of awareness regarding the safeguarding of the rights and welfare of the subjects.
- I. The ORA(s) will arrange for and document in its records that each individual who conducts or reviews human subject research has first been provided with a copy of this Assurance, as well as with ready access to copies of 45 CFR 46, regulations of other Federal departments or agencies as may apply, the Belmont Report, and all other pertinent Federal policies and guidelines related to the involvement of human subjects in research.
- J. The ORA(s) will report promptly to the IRB(s), appropriate institutional officials, the Office for Protection from Research Risks (OPRR), and any other sponsoring Federal department or agency head:
1. any injuries to human subjects or other unanticipated problems involving risks to subjects or others,
 2. any serious or continuing noncompliance with the regulations or requirements of the IRB, and
 3. any suspension or termination of IRB approval for research.
- K. The ORA(s) will ensure (a) solicitation (or confirmation where applicable assurances to comply already exist), receipt, and management of all assurances of compliance (whatever the appropriate format), and (b) certifications of IRB review (where appropriate) for all performance sites to this institution (including those listed in Appendix B) and subsequent submission of new documents to the proper Federal department or agency authorities (e.g., OPRR for DHHS) as a condition for involvement of each site in human subject research activities sponsored by DHHS or any other Federal department or agency for which this Assurance applies.
- L. The ORA will ensure that all affiliated performance sites that are not otherwise required to submit assurances of compliance with Federal regulations for the protection of research subjects at least document mechanisms to implement the equivalent of ethical principles to which this institution is committed (see Part 1, I).
- M. When an IRB of this institution accepts responsibility for review of research which is subject to this Assurance and conducted by any independent investigator who is not otherwise subject to the provisions of this Assurance, the ORA will either: (a) obtain and retain a Noninstitutional Investigator Agreement (NIA) for CPRP activities (with copy to the investigator and the authorizing CPRP) or (b) obtain an Agreement for an Independent Investigator (AII) for review

and approval by the appropriate Federal department or agency for non-CPRP activities to document the investigator's commitment to abide: (1) by the same requirements for the protection of human research subjects as does this institution(s) and (2) the determinations of the IRB(s).

- N. The ORA(s) assumes responsibility for ensuring conformance with special reporting requirements for any OPRR-recognized CPRPs in which the signatory institution(s) participate(s).
- O. The ORA(s) will be responsible for procedural and record-keeping audits not less than once every year for the purpose of detecting, correcting, and reporting (as required) administrative and/or material breaches in uniformly protecting the rights and welfare of human subjects as required at least by the regulations and as may otherwise be additionally required by this institution(s).
- P. The ORA(s) will ensure compliance with the requirements set forth in this Assurance and Section 114 regarding cooperative research projects. In particular, where the IRB of another institution with a DHHS MPA is relied upon, the ORA(s) will ensure that documentation of this reliance will be (a) in writing, (b) approved and signed by the ORA(s), (c) approved and signed by the correlative officials of each of the other cooperating institutions, and (d) retained by the ORA for at least three years past completion of the research project, if limited in scope to a specific research project or retained as a permanent addendum to the MPA if not restricted to a specific project. For all Cooperative Amendments (CAs), the ORA(s) will forward the original of the required signed understanding to OPRR for approval and inclusion in this Assurance as an addendum.

III. Institutional Review Board (IRB)

- A. The IRB(s) will review, and have the authority to approve, require modification in, or disapprove all research activities, including proposed changes in previously approved human subject research. For approved research, the IRB will determine which activities require continuing review more frequently than every twelve months or need verification that no changes have occurred if there was a previous IRB review and approval.
- B. IRB decisions and requirements for modifications will be promptly conveyed to investigators and the ORA, in writing. Written notification of decisions to disapprove will be accompanied by reasons for the decision with provision of an opportunity for reply by the investigator, in person or in writing.
- C. Initial and continuing convened IRB reviews and approvals will occur in compliance with 45 CFR 46 and provisions of this Assurance for each project unless properly found to be exempt (Section 101[b] or [i]) by the Office of Research Administration. Continuing reviews will be preceded by IRB receipt of appropriate progress reports from the investigator, including available study-wide findings.
- D. The IRB(s) will observe the quorum requirements of Section 108(b). This institution's IRB(s) has effective knowledge of subject populations, institutional constraints, differing legal requirements, and other factors which can foreseeably contribute to a determination of risks and benefits to subjects and subjects' informed consent and can properly judge the adequacy of information to be presented to subjects in accordance with requirements of Sections 103(d), 107(a), 111, and 116.
- E. The IRB(s) will determine, in accordance with the criteria found at 45 CFR 46.111 and Federal policies and guidelines for involvement of human subjects in HIV research, that protections for human research subjects are adequate.
- F. The IRB(s) will ensure that legally effective informed consent will be obtained and documented in a manner that meets the requirements of Sections 116 and 117. The IRB will have the authority to observe or have a third party observe the consent process.
- G. Where appropriate, the IRB(s) will determine that adequate additional protections are ensured for fetuses, pregnant women, prisoners, and children, as required by Subparts B, C, and D of 45 CFR 46. The IRB(s) will notify OPRR

promptly when IRB membership(s) is modified to satisfy requirements of 45 CFR 46.304 and when the IRB fulfills its duties under 45 CFR 46.305(c).

- H. Scheduled meetings of the IRB(s) for review of each research activity will occur not less than every 12 months and may be more frequent, if required by the IRB on the basis of degree of risk to subjects. The IRB may be called into an interim review session by the Chairperson at the request of any IRB member or institutional official to consider any matter concerned with the rights and welfare of any subject.
- I. The IRB(s) will prepare and maintain adequate documentation of its activities in accordance with Section 46.115 and in conformance with Office of Research Administration requirements.
- J. The IRB(s) will forward to the Office of Research Administration any significant or material finding or action, at least to include the following:
 - 1. injuries or any other unanticipated problems involving risks to subjects or others,
 - 2. any serious or continuing noncompliance with the regulations or requirements of the IRB, and
 - 3. any suspension or termination of IRB approval.
- K. In accordance with Section 113, the IRB(s) will have the authority to suspend or terminate previously approved research that is not being conducted in accordance with the IRB's requirements or that has been associated with unexpected serious harm to subjects.
- L. The IRB(s) for this institution will ensure effective input (consultants or voting or nonvoting members) for all initial and continuing reviews conducted on behalf of performance sites where there will be human research subjects. IRB minutes will document attendance of those other than regular voting members. The IRB list(s) in Appendix C includes those who are identified as knowledgeable about any affiliate institution having entered into an Inter-Institutional Amendment or other institutional performance site for which an Assurance is required when relying on one or more of the IRBs of this institution.
- M. The IRB(s) will act with reasonable dispatch, upon request, to provide full board review of protocols of OPRR-recognized Cooperative Protocol Research Programs (CPRP). The IRB will not employ expedited review procedures for CPRP protocols when they are to be entered into for the purpose of research. Although emergency medical care based on such protocols is permitted without prior IRB approval, patients receiving emergency care under these conditions will not be counted as research subjects and resultant data will not be used for research purposes.
- N. Certifications of IRB review and approval will be forwarded through the ORA to the appropriate Federal department or agency for research sponsored by such departments or agencies.

IV. Research Investigator

- A. Research investigators acknowledge and accept their responsibility for protecting the rights and welfare of human research subjects and for complying with all applicable provisions of this Assurance.
- B. Research investigators who intend to involve human research subjects will not make the final determination of exemption from applicable Federal regulations or provisions of this Assurance.
- C. Research investigators are responsible for providing a copy of the IRB-approved and signed informed consent document to each subject at the time of consent, unless the IRB has specifically waived this requirement. All signed consent documents are to be retained in a manner approved by the Office of Research Administration.
- D. Research investigators will promptly report proposed changes in previously approved human subject research activities to the IRB. The proposed changes will not be initiated without IRB review and approval, except where necessary to

eliminate apparent immediate hazards to the subjects.

- E. Research investigators are responsible for reporting progress of approved research to the Office of Research Administration, as often as and in the manner prescribed by the approving IRB on the basis of risks to subjects, but not less than once per year.
- F. Research investigators will promptly report to the IRB any injuries or other unanticipated problems involving risks to subjects or others.
- G. No research investigator who is obligated by the provisions of this Assurance, any associated Inter-Institutional Amendment, or Noninstitutional Investigator Agreement will seek to obtain research credit for, or use data from, patient interventions that constitute the provision of emergency medical care without prior IRB approval. A physician may provide emergency medical care to a patient without prior IRB review and approval, to the extent permitted by law (see Section 116[f]). However, such activities will not be counted as research nor the data used in support of research.
- H. Research investigators will advise the IRB, Office of Research Administration and the appropriate officials of other institutions of the intent to admit human subjects who are involved in research protocols for which this Assurance or any related Inter-Institutional Amendment or Noninstitutional Investigator Agreement applies. When such admission is planned or a frequent occurrence, those institutions must possess an applicable OPRR-approved Assurance prior to involvement of such persons as human subjects in those research protocols.

V. Affiliated Institutions and Investigators (i.e., all performance sites, with or without IIAs)

- A. Each performance site to this institution that is involved in federally sponsored research activities must provide to the Office of Research Administration an appropriate written assurance of compliance with the Belmont Report and the Federal Policy, to include Subparts B, C, and D or 45 CFR 46 where appropriate (or equivalent protections if a foreign site), for review and approval, as specified by the sponsoring Federal department or agency (e.g., by OPRR for DHHS), prior to involvement of human subjects or expenditure of funds or other support to do so.
- B. Each institutional performance site must respond to a request by the Office of Research Administration of this institution for an Inter-Institutional Amendment, SPA, or CPA (as appropriate), whichever is most suited to the circumstances.
- C. Each non-institutional performance site (e.g., a private practice physician not otherwise an employee of this institution or who otherwise would not ordinarily be bound by the provisions of this Assurance or any other applicable institutional Assurance) who is involved in human subject research of this institution must respond to a request by the Office of Research Administration of this institution for either an Agreement for an Independent Investigator or a Noninstitutional Investigator Agreement, as appropriate, depending on the nature of the research activity.
- D. Performance sites that are legally separable from this institution (whether an institutional or non-institutional performance site) are not authorized to cite this Assurance.

PART 3 - SIGNATURES

I. Institutional Endorsement(s)

The officials signing below assure that any research activity conducted, supported, or otherwise subject to DHHS or other Federal departments or agencies that are authorized to rely on this Assurance (Parts 1, 2, 3 and Appendices) or any other sources provided for in this Assurance, will be reviewed and approved by the appropriate IRB(s) in accordance with the requirements of all applicable Subparts of Part 46, Title 45 of the Code of Federal Regulations, with this Assurance, and the stipulations of the IRB(s).

A. Primary Signatory Institution (if any)

1. AUTHORIZED INSTITUTIONAL OFFICIAL

Signature: *James J. Corrigan, Jr.* Date: 11/23/98
Name: James J. Corrigan, Jr., M.D.
Title: Dean
Institution and
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1430 Tulane Avenue
New Orleans, La. 70112
Phone: (504) 588-5462
Fax: (504) 584-2945
E-Mail: jcorrigan@tmcpop.tcs.tulane.edu

2. PRIMARY CONTACT (If same, write "SAME")

Signature: _____ Date: _____
Name: SAME
Title: _____
Institution and
Address: _____

Phone: _____
Fax: _____
E-Mail: _____

FOR DHHS USE ONLY

II. Office for Protection from Research Risks (DHHS) Approval

A. DHHS RECOMMENDING OFFICIAL

Signature: Katherine Duncan Date: March 25, 1999
Name: Katherine Duncan, M.D.
Title: Adjunct Medical Officer
Address: Division of Human Subject Protections
Office for Protection from Research Risks (OPRR)
6100 Executive Boulevard, Room 3B01 (MSC 7507)
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Phone: 301-496-7005 X207
Fax: 301-402-0527
E-Mail: kd41f@nih.gov

EFFECTIVE DATE OF ASSURANCE: 3.30.99

EXPIRATION DATE OF ASSURANCE: 3.29.04

B. DHHS APPROVING OFFICIAL

Signature: Clifford C. Scharke Date: 3.30.99
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.

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1. REPORT DATE (DD-MM-YYYY) 07-31-2001		2. REPORT DATE Performance Technical Report		3. DATES COVERED (From - To) May 1999 - April 2001	
4. TITLE AND SUBTITLE Integrated Bioenvironmental Hazards Program				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER N00014-99-1-0763	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) McLachlan, John, Dr., PI Meffert, Douglas, Dr., Associate Director Wilson, Valerie, P., Deputy Director Kitzman, Helen, Dr., Project Administrator Johnson, Desiree, Senior Program Coordinator				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Tulane University 1430 Tulane Avenue, sl-3 New Orleans, LA 70112				8. PERFORMING ORGANIZATION REPORT NUMBER Xavier University of LA 1 Drexel Drive New Orleans, LA 70118	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research Ballston Centre Tower One 800 North Quincy Street Arlington, VA 22217-5660				10. SPONSOR/MONITOR'S ACRONYM(S) ONR	
				11. SPONSORING/MONITORING AGENCY REPORT NUMBER	
12. DISTRIBUTION AVAILABILITY STATEMENT APPROVED FOR PUBLIC RELEASE					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Beginning in April 1999, the Center for Bioenvironmental Research (CBR) at Tulane and Xavier Universities has received funding from the Office of Naval Research to continue its Bioenvironmental Hazards Research Program (BHRP). This funding has supported a suite of complementary research projects that address the impacts of bioenvironmental hazards on environmental signaling from molecular to ecosystem levels and makes connections between these impacts. The research ranges from basic research on proteomics to applied technology development of biosensors and autonomous underwater vehicles for monitoring. The BHRP program also includes mechanisms for the effective communication of this information for resolution of Department of Defense problems and for the educational training of future scientists. Seventeen research projects have been conducted in the two primary research modules and have resulted in significant progress related to the overall grant objectives. This program reflects the CBR's existing research strengths and employs a set of integrated research modules to assess the impacts of "environmental signals" (e.g., contaminants and pollutants) on humans and ecosystems.					
15. SUBJECT TERMS Autonomous Underwater Vehicles (AUV), Biosensors, Communication & Education, Environmental Signaling					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES 197	19a. NAME OF RESPONSIBLE PERSON Dr. Valerie Wilson, Deputy Dir.
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER (Include area code) (504) 585-6910