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**Tulane/Xavier Universities
Center for Bioenvironmental Research
Hazardous Materials In
Aquatic Environments
Of The Mississippi River Basin**

Quarterly Progress Report

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Project #DE-FG01-93EW53023
(July 1, 1996 - September 30, 1996)

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RE: Hazardous Materials in Aquatic Environments of the Mississippi River Basin
Contract #DE-FG01-93EW

Enclosed are two copies of the Quarterly Project Status Report for the period July 1, 1996 - September 30, 1996 for the above referenced grant.

If you need any additional information, please contact me at (504) 865-5639.

Sincerely,

Verna Lee HC

Verna Lee
Program Manager

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ADMINISTRATIVE ACTIVITIES

Hazardous Materials in Aquatic Environments of the Mississippi River Basin Project Management

J. McLachlan, C. F. Ide, S. O'Connor

Objectives and Goals:

To provide the necessary administrative support to assure that the scientific and educational goals of the project are obtained and to assure that all Department of Energy reporting requirements and requests are fulfilled. The grant reporting is divided into four aspects: Collaborative Cluster research projects, Initiation research projects, Technical Support activities and Education/Research Training activities.

A collaborative cluster project is one or more closely related collaborative, multidisciplinary research projects in which a group of investigators employs a synergistic approach to the solution of problems in the same general area of research.

An initiation project typically involves a single investigator. The purpose of the project is to undertake pilot work, lasting no more than one year, which will lead to the successful submission of an externally-funded proposal or the development of a collaborative cluster project.

Technical support activities involve anything that is required to support the competitively funded research and the overall grant objective of characterizing the identified field sites. Core groups have been established for each of the following areas: field work, chemical analyses, data management, and research training and education. The core approach involves the establishment of flexible teams of researchers who will not only provide support for this project, but are part of the development of an infrastructure which will be utilized to support the Tulane/Xavier Center for Bioenvironmental Research's (CBR) expanding environmental research efforts.

The research training and education core is designed to develop courses with emphasis on environmental studies and/or to train students in areas of environmental research.

Accomplishments This Quarter:

- A Planning/Review meeting was held July 29-30, 1996. The purpose of the meeting was to highlight the on-going research of this grant. The meeting was attended by 135 people. Attendees included Jef Walker and Madhav Ghate from DOE, several of the scientists who serve as reviewers/consultants to the grant, as well as the grant's researchers.

The research presentation portion of the Planning/Review meeting was open to the public. Three of the local television stations interviewed participants. Additionally, there were guests from state agencies and local environmental groups.

- The administrative staff provided fiscal data in response to special requests from the DOE program management.
- Work continued on the long term research objectives for this project including the development of a grant proposal for continuation funding for 1998-2002.
- Dr. John McLachlan and other Tulane and Xavier officials met with Mr. Al Alm and Dr. Clyde Frank at DOE headquarters to discuss DOE research needs and CBR capacities, as well as to report on the ongoing Tulane/Xavier partnership.
- The quarterly progress report for the second quarter of 1996 was submitted to DOE.
- Dr. Charles F. Ide Dr. Sally O'Connor and Ms. Verna Lee attended the Spectrum '96 Conference: International Topical Meeting on Nuclear and Hazardous Waste Management. The meeting was held August 18-23, 1996 in Seattle Washington.
- Dr. John McLachlan, Dr. Sally O'Connor and Ms. Verna Lee met with Madhav Ghate, the DOE program manager, and others at the Morgantown Energy Technology Center. This meeting provided an opportunity for the grantees to meet DOE personnel and to plan for future endeavors.

Tulane/Xavier Center for Bioenvironmental Research

J. McLachlan, C. F. Ide, A. Reine

Objectives and Goals:

The Tulane/Xavier Center for Bioenvironmental Research (CBR) is an interdisciplinary research center dedicated to investigating the causes and effects of environmental problems and devising practical solutions for them. The CBR fosters extensive collaborative research with such diverse disciplines as toxicology, medicine, epidemiology, cell and molecular biology, chemistry, ecology, engineering, and information sciences. Working together, scientists in these areas can draw on each other's strengths, providing a much greater opportunity for unraveling complex biomedical problems.

Faculty associated with the CBR study both health effects and remediation techniques, with the ultimate goal of developing responsible public policy. Research focuses on understanding the basic mechanisms by which toxic substances compromise health. This allows scientists to design prevention, intervention, and therapeutic strategies to mitigate the effects of human and animal exposure to environmental contaminants. Such research will also lead to new technologies for the removal and degradation of toxic materials from contaminated areas. Most importantly, environmental research is the cornerstone of effective strategies for disease prevention.

The CBR promotes the use of science in the formulation of public policy and offers expert communication about environmental health risks and their management. To achieve these goals, the CBR is establishing an active partnership with business, government, and interested members of the public in order to make the CBR's research responsive to the needs of the entire community. The CBR is particularly concerned with resolving environmental issues facing Louisiana and the Gulf South region.

Accomplishments This Quarter:

Research

- Two large grant proposals were funded. An additional proposal was submitted.
- Committees have been formed to recruit two junior faculty jointly between Xavier and Tulane Universities, to work in the area of molecular modeling with a focus on hormonal activity of environmental chemicals. These faculty will be supported by an NSF/EPSCoR Joint Faculty Appointment Program grant.
- Several high profile papers have been published by the CBR's new research unit, environmental endocrinology.

Information Science

- The CBR's "Environmental Concepts Made Easy", an innovative web site designed to provide accurate and timely information on featured environmental topics, has been broadly promoted to over 2,000 scientists, journalists and educators. Several university classes across the country have been taught using this interactive site to conduct research and discuss via internet the topics with research investigators. <<http://www.tmc.tulane.edu/ECME/>>.
- A proposal submitted in September to the W. Alton Jones foundation would expand the ECME program.

Research Collaborations -- Strengthening the Partnership

- Construction is underway on the new Research and Teaching Laboratory for Environmental Biotechnology. The lab has been designed to enhance research capabilities for clinical and basic science. A committee has been established to identify one Xavier faculty member and student to rotate through the Tulane-based lab starting in January, when the lab construction is completed.
- The CBR's formal membership, initiated in June, now has recruited 50 of the best research faculty from Tulane and Xavier.

COLLABORATIVE CLUSTER PROJECTS

A Sensitive Rapid On-Site Immunoassay for Heavy Metal Contamination

R. Blake, D. Blake, G. Flowers

Objectives and Goals:

The principal goal of this focus group is to develop immunoassays for heavy metals that will permit the rapid on-site analysis of specific heavy metals in water and soil samples. Previous studies have shown that monoclonal antibodies to specific metal-chelate complexes may be obtained that permit the quantification of indium in the ppb range even in the presence of excess concentrations of other metals. This group seeks to extend these studies to develop immunoassays for target metals that include, but are not limited to, lead and chromium. Specific aims include: 1) to develop new immunoassays for metals that are priority pollutants; 2) to validate each new metal immunoassay by comparing the results with those obtained by atomic adsorption spectrometry and inductively coupled plasma spectrometry; and 3) to test these new assays on samples collected at previously studied test sites and to initiate the development of field applications for the immunoassay.

Accomplishments This Quarter:

- Antibodies directed against ionic lead have been achieved

Ten milligrams of a monoclonal antibody directed against an epitope of Pb(II) chelated with a bifunctional derivative of cyclohexyl-diethylenetriaminepentaacetic acid (CHA-DTPA) have been isolated. The hybridoma that produced the antibody was cloned twice by limiting dilution and was shown to stably secrete an IgG-type antibody. Preliminary binding studies performed with the KinExATM automated immunoassay instrument revealed that the structure of the chelator had a strong influence on the affinity of the antibody for the metal-chelate complex. The antibody affinity increased in the following order: DTPA-Pb(II) < 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid-Pb(II) < CH-DTPA-Pb(II) (the soluble form of the cyclohexyl-DTPA). Ionic lead chelated with CH-DTPA was readily detected in the low nanomolar range in the KinExATM, while a comparable instrument response required low micromolar concentrations when the lead was chelated with DTPA that lacked the cyclohexyl moiety. Binding studies to characterize the metal specificity of the antibody in the presence of different chelators are in progress. In the meantime, competitive ELISA's were performed with this antibody in the presence of fixed concentrations of different metal cations. The lead immunoassay was insensitive to interference's by other metal cations with the exception of Ca(II), which was a very strong competitor for the antibody binding site. The sensitivity of the lead immunoassay was enhanced 5-fold by substituting DTPA-lead for CH-DTPA-lead on the solid phase, a strategy that rendered the soluble DTPA-Pb(II) a better competitor in the assay.

- Hybridoma production is underway at Tulane

Due to difficulties with the external supplier, hybridoma generation and production are being conducted in the research laboratory at Tulane. An appropriate animal care protocol was approved, and immunization of mice with a protein conjugate of DTPA-Pb(II) without the cyclohexyl group was initiated. The binding studies summarized above suggested that the cyclohexyl group was sufficiently immunodominant to overshadow the metal-chelate portion of the epitope. It is anticipated that the ability to perform and control every step in the production of new monoclonal antibodies will enhance our opportunities to explore new and novel bifunctional chelators that may favor particular metal cations.

- Construction of a field-portable immunoassay for cadmium

The validity of the prototype immunoassay for Cd(II) was tested by comparing the results obtained by ELISA with those obtained by the corresponding atomic absorption spectrometric measurements. Analyses for Cd(II) were conducted by both methods in each of 30 different spiked water samples. These samples included both reagent grade water and natural samples from Bayou Trepagnier. Bayou Trepagnier is a polluted wetlands system near New Orleans which this grant has targeted for study. Discrepancies between the 2 methods noted previously were resolved in the current work by careful QA/QC experiments performed in the laboratories of Drs. Blake and Flowers. The latest comparisons indicated that the values for Cd(II) as determined by ELISA were linearly correlated with those determined by atomic absorption with a slope of 0.951 and a correlation coefficient of 0.931. A manuscript is in preparation for Environmental Science and Technology that describes these results.

Publications and Presentations:

Blake, D.A., Chakrabarti, P., Khosraviani, M., Hatcher, F.M., Westhoff, C.M., Goebel, P., Wylie, D.E., and Blake, R.C. II. Metal Binding Properties of a Monoclonal Antibody Directed Towards Metal Chelate Complexes. *Journal of Biological Chemistry*. (in press)

Blake, D.A., Pavlov, A.R., Khosraviani, M., and Blake, II, R.B. "Determination of antibody affinity and specificity", *Proceedings of the Immunochemistry Summit V and First Joint Conference on Immunochemistry*, August 13-15, 1996 Las Vegas, NV

Flocculation Studies In Environmental Processes

E. Eschenazi , B. Das, R. Effiong

Objectives and Goals:

The objective of this project is to study flocculation phenomena in suspensions and emulsions, resulting from the process of aggregation of suspended particles. Flocculation is not only a determining factor in natural environmental processes, but also constitutes a tool for the cleaning of hazardous waste waters which contain suspended solids and emulsified oils. Understanding flocculation kinetics in various regimes is crucial to design proper remediation. The project is a combination of experimental and theoretical studies of two physicists and one chemist from Xavier University.

Experimentally

Multi-angle Light Scattering

One of the fundamental experimental techniques being used to study flocculation kinetics is light scattering. This technique allows the measurement of the particle size distribution (PSD), its temporal evolution as function of heavy metal concentration as well as pH and concentration of indifferent ions (e.g. K^+ and Cl^-).

Analysis of Metals in the Emulsions

Divalent ions specifically adsorb on the surface of suspended particles, thus playing a pivotal role in promoting coalescence. It is therefore important to know how certain metals such as Ni^{++} and Pb^{++} partition between the bulk aqueous solution and the surface of the emulsion droplets before and after the aggregation. Techniques such as Atomic Absorption Spectroscopy (AAS), Fourier-Transform Infrared Spectroscopy (FTIR) and Inductively-Coupled Plasma Spectroscopy (ICP) are used to trace metals in the solutions.

Theoretically

At the *theoretical level*, studies of the aggregation kinetics utilizing computer simulation techniques continued. In particular a Monte-Carlo technique is being used to investigate the collisional probabilities in concentrated emulsions. The experimental results will be compared with the calculations to develop a consistent predictive tool for flocculation kinetics in various regimes.

Accomplishments This Quarter:

1. Samples preparation:

- Samples of n-hexadecane/water emulsions with and without surfactants were prepared by Dr. R. E and her student, Chris J. Watts . Emulsions containing three kinds of surfactants prepared , namely anionic, cationic and neutral. Some of the emulsions were contaminated with chromium and the pHs adjusted. The remainder were left uncontaminated to use as controls for the experiments. At chromium concentrations of less than 200 ppm, both anionic and cationic surfactants containing emulsions show great stability. This is seen in the constancy of the particle size. Recent research endeavor has shown that at chromium concentration of 300 - 500 ppm, CTAB (cationic) surfactant containing emulsions showed varying degrees of instability. The chromium content of the "sediments" in aqueous media be analyzed. An interesting finding was that the anionic surfactant containing emulsion was stable at above mentioned chromium concentrations.

2. Multi-angle-Light scattering experiments

- Dr. Das and his student, Jameel Uqdah, continued the experimental studies of flocculation processes using the computerized laser light scattering apparatus. The effects of pH and the effects of chromium metal ion concentrations on the stability of emulsions and flocculation's over a period of time are being studied. A constant temperature bath was purchased to keep the emulsion at constant temperature while taking the particle size measurements. Since the viscosity of the medium, which is a function of temperature, affects the motion of the particles in the medium, the temperature of the emulsions need kept constant through out the measurements. In particular, the experimental studies of change of pH (3.3 to 6.8) of the emulsion and addition of 10 ppm and 100 ppm chromium in the hexadecane-in-water emulsion has shown the particle size change from 175 to 190 nm for the emulsion with added cationic surfactant and a size change from 235 nm to 275 nm for the emulsion with added anionic surfactant. These measurements did not show any evidence of flocculation in the system due to added chromium contaminants. Preliminary results of adding chromium concentration of 500 ppm shows more increase in the average particle size (about 1.3 time the particle size compared to the pure emulsion with added surfactant) which is likely to be because the addition of chromium in the medium and we are investigating it further. Through experimental measurements, particle sizes and any evidence of flocculation using higher concentration of chromium ion as well as using added concentrations of cadmium, zinc, nickel and lead in the emulsions.

3. FTIR studies:

- Dr. Akundi and his student Aghenia Tsega conducted FTIR spectral studies on emulsions containing cationic and anionic surfactants.

4. Monte-Carlo and numerical simulations

- Dr. Eschenazi continued the numerical study of the collisional probabilities in concentrated emulsions using Metropolis algorithm. In collaboration with Dr. K. Papadopoulos, Dr. S. Deshiikan Rangaraj Dwana Bush at the department of mechanical engineering, at Tulane University, the effects of surfactant on surface tension are being studied. (A paper will be submitted at the end of October.)

Publications and Presentations:

Deshiikan, S. R., Bush, D., Eschenazi, E. V., Papadopoulos, K. Brij58, CTAB and SDS at the Slowly Dilating n-Dodecane-Water Interface. Colloids and Surfaces (Submitted)

Pore-Level Flow, Transport, Agglomeration and Reaction Kinetics of Microorganisms

L. Fauci, D. Gaver, K. Medjani, P. Moore, K. Papadopoulos

Objectives and Goals:

Understanding the detailed pore-level behavior of microorganisms through porous media is essential for the development of effective *in situ* bioremediation strategies. This group is developing integrated experimental and computational models of the pore-level behavior of microorganisms. The models include the detailed analysis of convection and diffusion of contaminant and microorganisms within micropores. In addition, these studies include chemotactic responses of swimming microorganisms to local concentration gradients. Microorganisms modify the pore structure due to adhesion to each other and the surrounding pore. These models incorporate reaction kinetics of these organisms to the toxic contaminant. The primary goal is to identify physicochemical characteristics that influence microbial transport and uptake in toxic environments.

Accomplishments This Quarter:

- Stephanie Kute, previously an undergraduate studying basic models of aggregation, was awarded an NSF graduate fellowship to continue these studies at Tulane. Ms. Kute has implemented boundary element code into her models of aggregation. This code permits the analysis of a much larger number of bacteria than the studies by Dr. Dillon, but does not incorporate bacterial swimming behavior into these biofilm studies. She is modeling the influence of fluid flow induced shear stresses on biofilm formation and has found that an extremely large shear stress must be sustained to support monolayer formation. Ms. Kute will defend her Master's thesis on this topic in October.
- Dean Bottino, a graduate student in Mathematics at Tulane University, successfully defended his Ph.D. dissertation, *A Model of Ameboid Deformation and Locomotion*. In September, he began an instructorship position in the Department of Mathematics at the University of Utah.
- Dr. Lisa Fauci was an invited speaker at the *Second International Congress of Nonlinear Analysts* in Athens, Greece in July, 1996. She presented this project's work on biofilm modeling.
- Dr. Donald Gaver was an invited speaker at the *Biomedical Engineering Society Annual Meeting* in State College, Pa, where he presented analyses of coupled physicochemical processes in biofluid mechanics problems relevant to biofilm formation.
- Zewen Liu began postdoctoral work to continue investigations on bacterial motility in restrictive capillaries in the Department of Chemical Engineering at Tulane University.
- Robert Dillon, a postdoctoral researcher on this project, has been awarded an NSF postdoctoral fellowship to continue these studies at Tulane. During the most recent quarter, he implemented studies of microbial growth in biofilm models.

- Dr. Khoudir Medjani, Professor of Mechanical Engineering at Xavier University continued work on transport phenomena in porous media.
- Four graduate students and five undergraduate students are working on various aspects of this research. The graduates are Mr. Dean Bottino (Mathematics), Mr. Zewen Liu (Chemical Engineering), Mr. Darren Yap (Biomedical Engineering), Mr. Dave Meng (Mathematics). The undergraduates are Jeff Fredrichs (Mathematics), Kristen Swanson (Mathematics) and Brandon Downey (Mathematics), Darryl Overby (Biomedical Engineering) and Molly Charlton (Chemical Engineering).

Publications and Presentations:

Dillon, R., Fauci, L., Fogelson, A. and Gaver, D. Modeling Biofilm Processes Using the Immersed Boundary Method. *Journal of Computational Physics*. (Accepted)

Dillon, R., Fauci, L., and Gaver, D. A Microscale Model of Bacterial Swimming, Chemotaxis and Substrate Transport. *Journal of Theoretical Biology*. 177:325-340, 1995.

Liu, Z. and Papadopoulos, K. Chemotaxis In Near-Linear Gradients Of Chemoattractants. *Biotechnology and Bioengineering*. (In review)

Liu, Z., Chen, W. and Papadopoulos, K. Bacterial Motility, Collisions and Aggregation in 3mm-diameter Capillary. *Biotechnology and Bioengineering*. (in press)

Liu, Z., Chen, W. and Papadopoulos, K. Cell Starvation Effects on Bacterial Transport Through Sand-Packed Columns. *Environmental Sciences and Technology*. (in review)

Liu, Z. and Papadopoulos, K. A Method for Measuring Bacterial Chemotaxis Parameters in a Microcapillary. *Biotechnology and Bioengineering*. (51:120-125, 1996).

Moore, P. and Dillon, R. A Comparison Of Several Preconditioners In The Solution Of Parabolic Systems In Three Space Dimensions Using DASP6 And A High-Order Finite Element Method. *Applied Numerical Mathematics*. (Accepted)

Biomarkers of Exposure and Ecotoxicity in the Mississippi River Basin

W. Hartley, T. Huang, C. Ide, M. Mizell, P. Obih, A. Thiyagarajah, R. Tompkins

Objectives and Goals:

The goals of the project are: 1) to determine which biomarker responses reflect the combined toxicological interactions in frogs and fish exposed to mixtures of environmental contaminants typical of Devil's Swamp and Bayou Trepagnier; 2) to determine which biomarker responses integrate exposure over-time (biomarker responses integrating exposure over-time may indicate subacute, subchronic and chronic adverse ecological effects of mixtures of contaminants); and 3) to identify and characterize the most sensitive sentinel species based on biomarkers.

The specific hypotheses are: 1) histopathological response is a valid biomarker for exposure over time and the toxicological effects of mixtures of contaminants; 2) heavy metal and organic pollutants cause abnormal development and physiology in frogs in controlled laboratory tests; 3) pollutants in water and sediment samples will correlate with accumulations in tissues; 4) pollution levels in tissues will correlate with expression of biomarkers related to altered function in embryonic development, immune responses, and neural function; and 5) esterase enzymes are effective biomarkers of exposure in fish and frogs.

Accomplishments This Quarter:

Histopathology of Fishes from Devil's Swamp and Tunica Swamp

- There were two sampling trips to Bayou Trepagnier and Bayou Traverse.
- A manuscript on the neoplasms in bowfin from the Mississippi River Basin is currently being prepared.
- Histopathological examination of fish species other than gar and buffalo collected from Devil's Swamp and Tunica Swamp, the control site, is in progress.
- A manuscript on melanomacrophage centers in the liver of gar fish from Devil's Swamp is being prepared.
- Gonadal cysts were noted in gars from Bayou Trepagnier. Evaluation of the etiology of these cysts, particularly ovarian cysts is in progress.

Metabolism and Neurotoxicology

- Esterase activities (AChE and CaE) in the liver of spotted gar were significantly lower in fish from Bayou Trepagnier compared to the control fish from Tunica Swamp.
- Esterase depression was greater in the gars from Bayou Trepagnier than in the gars from Devil's Swamp.
- The esterase activities were significantly lower in the liver of the carp from Devil's Swamp compared to those from Tunica Swamp.

Publications and Presentations:

Major, S., Thiagarajah, A., Hartley, W. R. and Broxson, M. Image Analysis of Presumptive Mast Cells in the Gills of Bigmouth Buffalo Fish, *Ictiobus cyprinellus*, as a Biomarker of Exposure to Xenobiotics was accepted for presentation at the 17th annual meeting of the Society of Environmental Toxicology and Chemistry, Washington, D.C., November 17-21, 1996.

Jaiswal, R., Huang, T., Obih, P., Igbo, I., Hartley, WR., and Thiyagarajah, A. 1996. Esterases as potential biomarkers for environmental risk assessment. To be presented at the 17th annual meeting of the Society of Environmental Toxicology and Chemistry, Washington, D.C., November 17-21.

Natural and Active Chemical Remediation of Toxic Metals, Organics, and Radionuclides in the Aquatic Environment

G. McPherson, G. Flowers, R. Gonzales, V. John, S. O'Connor, P. Pintauro, J. Zhang

Objectives and Goals:

This is a cluster whose research efforts are directed towards studying chemical aspects of remediation. Among topics in natural remediation are the fate of heavy metal and organic contaminants discharged into aquatic environments, and the development of new technology to accurately assay metal contaminants partitioned into soils, water, and tissue. Among topics in active remediation are the development of novel polymeric membranes and microporous solids for the entrapment of heavy metals, and the development of hybrid chemo-enzymatic oxidative schemes for aromatics decontamination.

Accomplishments This Quarter:

Subproject I: Metal-Sediment Interactions at the Fresh-Salt Water Interface

Efforts have been concentrated on the study of the partitioning of cadmium and lead ions in solution between free metal ions and those which are complexed with humic and fulvic acid. An ultra-filtration technique is being used to perform these experiments. Results to date are as follows:

- When the concentration of humic acid is increased a sharp decrease in the adsorption of the metal is observed. In the presence of 0.1 M NaNO₃, this decline in adsorption is not apparent.
- A sharp drop in the adsorption of humic acid with increasing pH was observed.
- An interesting observation is that as the concentration of sodium chloride is increased, lead adsorption (or removal from solution) increases while humic acid removal decreases.

Subproject II: Enzymatic Decontamination of Organic Wastestreams

The finding that trace amounts of anionic surfactants significantly modify the chemical structure of the polymeric species. With no surfactant, the product of enzymatic reaction is primarily composed of dimeric ketonic units (Pummerer's ketone). With low levels of surfactant, the product distribution changes to polyphenolics with higher molecular weights. Other aspects of the work include:

- Structural characterization of the polymer through IR and NMR and GC/MS.
- Characterization of enzyme viability during the process. Loss of enzyme through adsorption to the polymer.
- A graduate student's master's thesis will be on this topic.

Subproject III: Heavy Metal Chemistry of Bayou Trepagnier

- QA/QC analysis of data collected during previous field season has been completed.
- Soils found near the spoil bank crest (5 to 6 feet above the bank) are highly contaminated with lead throughout the study area. The most contaminated sample taken in the profile contains 7.6% or 76,000 ppm lead by weight.
- Samples collected along Transect 1 indicate that lead was transported from the spoil bank to Engineer's Canal (353 feet from the crest) and over 995 feet into wetlands adjacent to the bayou.
- A similar pattern is observed for chromium and zinc in soil samples taken along all nine transects.

Subproject IV Metal-Ion Sequestering Polymers

The effect of varying the alkyl phenoxy side groups of phosphazene polymers on the rate and extent of solid-state polymer photo-crosslinking, using UV light and benzophenone as the photoinitiator is under investigation. The phosphazene polymers contained either methylphenoxy, ethylphenoxy, or isopropylphenoxy side chains for crosslinking, with the alkyl group in either the meta or para position. Specific results follow:

- The reaction rate for photochemical crosslinking was found to vary with the alkyl substituent and followed the order methyl > ethyl > isopropyl.
- The same alkyl group ordering was observed from crosslinked polymer that were swollen in dimethylacetamide. Less polymer swelling provided indirect evidence of more crosslinks. Methyl phenoxy-substituted phosphazenes exhibited the lowest swelling and isopropylphenoxy phosphazenes the highest.
- Evidence of crosslinking was also observed by a rise in the glass transition temperature of the crosslinked polymers. The glass transition temperature increased with increasing amounts of benzophenone photoinitiator in the polymer. the highest glass transition temperatures were observed with methylphenoxy-substituted phosphazenes.
- Alkyl groups in the meta position on the phenoxy rings of phosphazene polymers exhibited greater chemical stability during photo-crosslinking (less degradation) than the corresponding alkylphenoxy-substituted phosphazenes with the alkyl group in the para position.

Subproject V: Role of Dissolved Organic Carbon in Metal Transport

Heavy metals such as lead, copper, chromium and zinc are particle-reactive pollutants that have been shown to be strongly linked to the carbon cycle in aquatic systems. These heavy metals, which are associated with resuspended particulate organic matter (POM; $> 0.2 \mu\text{m}$) and high molecular weight dissolved organic matter (HMW DOM; $< 0.2 \mu\text{m}$ and $> 1 \text{ kDa}$), can be readily transported with currents, tides, and wave action in the Mississippi River Basin.

- Based on sampling of three stations across Lake Pontchartrain, concentrations in HMW DOM for lead ranged from 5.68 to 11.53 ppb, copper ranged from 0.91 to 1.04 ppb, chromium ranged from 3.34 to 7.84 ppb, and zinc ranged from 0.89 to 4.77. The highest concentrations of lead and chromium occurred near Manchac Pass while the highest copper and zinc concentrations were found in the middle of the lake.
- In Bayou Trepagnier, where a history of petrochemical dumping has occurred, lead ranged from 0 to 0.55 ppb in the dissolved phase ($< 0.2 \mu\text{m}$), 1.81 to 13.39 ppb for the particulate phase, and below detection limits in the truly dissolved ($< 1 \text{ kDa}$). The concentrations of other metals were as follows: copper ranged from 0.19-1.08 ppb (dissolved), 22.07-70.01 ppb (particulate), and 0-0.51 ppb (truly dissolved); chromium ranged from 0-14.26 (dissolved), 6.30-10.17 ppb (particulate), and 0-10.3 (truly dissolved); zinc ranged from 4.06-7.44 (dissolved), 28.28-38.55 ppb (particulate) and 2.77-13.36 ppb (truly dissolved).
- Partitioning coefficients ($\log K_d$; ratio of dissolved metal concentrations with respect to particulate metal concentrations) ranged from 0 to 6.1 for lead, 6.4 to 6.9 for copper, 0 to 6.5 for chromium, and 5.4 to 5.7 for zinc in Bayou Trepagnier, suggesting that these heavy metals can be mobilized in the dissolved phase; this was most likely associated with HMW DOM.

Collaborative Research With the Institute of Radioecological Problems (IREP) - Fate and Transport of Radionuclides in Belarus Following the Chernobyl Catastrophe

E. Michaelides, E. Eschenazi, C. Koutsougeras,
S. Ramer, D. Sailor, L. Steinberg

Objectives and Goals:

To formulate the problem of aquatic transport in the Iput River, and train the two Byelorussian students in the numerical methods of transport of radionuclides and apply the knowledge gained from this project to the transport of sediment and pollutants in aquatic environments in Louisiana. The Byelorussians are graduate students at Tulane University School of Engineering.

Accomplishments This Quarter:

- The calibration process on the transport of sediment for the HEC-6 code has been completed. Work for the transport of cases using the code has commenced. The results on the transport of sediment in the Iput river for the next twenty years were received. Much of this data was transmitted to Xavier for visualization studies.
- Dr. Yassuo Onishi of Pacific Northwest Lab gave a lecture and explained the functions of his own transport program. As a result of this visit and discussions with Dr. Onishi, it is expected that this group will collaborate with PNL scientists in the future.
- An abstract has been accepted for the ASME "Liquid-solid Flows" symposium to be held in the summer of 1997. The paper for this symposium is in the preparation stages.
- Drs. Steinberg and Luna continued work on the aquatic transport in the Bayou Trepagnier. Mr. Pimenov, a graduate student, is currently reading the pertinent literature and experimenting with the Dyn-Hyd part of the WASP-5 program, for the modeling of the Bayou.
- Professors Sailor and Eschenazi continue their work on the modification of the code module on turbulence.

Publications and Presentations:

A presentation of our work was given the International Conference on "The Impact of Nuclear Power Plants and other Radiation," in Obninsk, Russia, sponsored by UNESCO.

INITIATION PROJECTS

Molecular Mechanisms of the Developmental Toxicity Induced by Retinoids and Retinoid-like Molecules

C. A. Burdsal

Objectives and Goals:

Teratogens are agents that interfere with the process of embryonic development and cause malformations and other anatomical defects. Retinoids, like steroids, are small lipophilic molecules that are passively transported through the cell membrane. Retinoid molecules bind to receptors and induce target gene expression. Accordingly, any molecule that can mimic retinoid action may activate target gene expression and be a dangerous environmental teratogen. Recently, the pesticide, methoprene, and its derivatives have been shown to influence transcription in vertebrates by directly activating one class of the retinoid receptors. The aims of this study are 1) to determine if molecules with retinoid activity are present in aquatic environments of the southern Mississippi River basin and; 2) to investigate the mechanism by which retinoids induce teratogenesis during mouse embryonic development.

Accomplishments This Quarter:

- Assays were underway in which a mammalian cell line that can detect the presence of retinoids was used. These cells are called *RARE-lacZ* cells. In response to retinoids, these cells express a protein called lac Z or (b-galactosidase) which can be easily detected histologically. Because retinoids can freely enter any cells they come in contact with, these cells can be used as an indicator of the presence of retinoids in water and extracts from sediment samples. Experiments were begun to test for the presence of retinoids and retinoid-like molecules in samples collected from Bayou Trepagnier and to identify sites of retinoids that are proposed to occur naturally in the mouse embryo. To date, no significant levels of retinoids have been detected in either set of experiments. These assays will be continued in next quarter.

Development of Cytochrome P450 Isozymes with Novel Designed Catalytic Activities for Use in Bioremediation of Recalcitrant Organic Pollutants

D. Mullin

Objectives and Goals:

The goal of this project is to develop enzyme catalysts that will enhance the natural ability of bacteria to degrade recalcitrant environmental pollutants like pyrene and tetrachloroethane. Protein engineering methods were used to develop an enzyme that oxidizes pyrene, and another enzyme that dechlorinates tetrachloroethane. The next step will be to test the ability of these enzymes to facilitate the complete mineralization of pyrene and tetrachloroethylene in the bacterium *Caulobacter crescentus*.

Background

The cytochromes P450 are a class of heme-containing enzymes with diverse biological functions that range from intermediary metabolism and development to roles in detoxifying and eliminating environmental pollutants from biological systems. More than 100 P450 enzymes have been characterized at the molecular level, and these oxidize a variety of aliphatic and aromatic compounds. One feature that all P450 enzymes share is the presence of a thiolate coordinated b-type heme which is the reactive center of all such enzymes, and thus, the substrate specificity and reactivity of a particular enzyme results from the unique geometry and chemical character of the substrate binding site which is determined by the amino acid sequence of a particular polypeptide. It therefore seems to be a reasonable assumption that by making appropriate changes to the amino acid sequence of a particular P450 enzyme, one should be able to change its substrate specificity and reactivity.

This project's experimental approach uses site directed mutagenesis to introduce specific alterations in the amino acid sequence of the cytochrome P450 102 of *Bacillus megaterium* in order to reshape the substrate binding channel and active site. P450 102 is especially useful for the purpose of this project because the P450 monooxygenase and heme reductase activities are carried in the same polypeptide chain whereas virtually all other known P450s require a separate P450 reductase protein. The required P450 reductase activity is thus, automatically co-introduced with the oxidase. This last feature should facilitate the expression of P450 102 enzymes with interesting activities in *C. crescentus*.

The investigation of how the natural substrate (long chain fatty acids) binds to wild type and mutant P450 102 isozymes helped to guide us in changing it from a fatty acid hydroxylase into a polycyclic aromatic hydroxylase that hydroxylates pyrene and benzo[a]pyrene which are environmental pollutants. These results demonstrate that protein engineering methods can be used to dramatically change the substrate specificity of P450 102, and it suggests that it should be possible to create designed isozymes that have other desired catalytic activities.

Accomplishments This Quarter:

- Progress continues in the dissection of the substrate binding domain of cytochrome P450 102 using site directed mutagenesis. The role of three additional amino acid residues in substrate recognition are being tested. One residue that is particularly interesting to us is M5 which is on the outer surface of the protein far away from the substrate binding channel. The mutagenesis data suggests that M5 may play an important role in the catalytic cycle of P450 102 and much of our attention this quarter has focused on the role of M5 in the catalytic cycle. It was found that M5R mutants have normal spectroscopic properties, but they fail to bind to laurate whereas the wild type enzyme is a very active laurate hydroxylase. M5T, and M5V mutants on the other hand express very active laurate hydroxylase activities, although their substrate preferences are somewhat different from the wild type enzyme.
- Using the crystal structure of the P450 102 heme domain (3) as a starting point, computer-aided molecular modeling was used to obtain a quantitative understanding of the biochemical properties of the M5R mutant enzyme. Preparation of a manuscript describing the M5 mutations is underway.
- Work is underway to construct a *Bacillus megaterium* strain in which the P450 102 gene in the chromosome has been inactivated (a knock-out mutant). The phenotype of this knock-out mutant should give us some clues as to the natural function of P450 102.

Publications and Presentations:

Xia, Q., Kang, L., Alworth, W. L., Liu, H. M., Zhao, W., and Mullin, D. A. A site specific mutant of the bacterial cytochrome P450 102 (BM-3) possessing a new capability to catalyze the hydroxylation of polycyclic aromatic hydrocarbons Pyrene and Benzo[a]pyrene. Archives of Biochem. Biophys. (Submitted)

Zhao, W. and Mullin, D. A. Probing the Structure and Function of Cytochrome P450 102 by Site Directed Mutagenesis: an Amino Acid Substitution of Valine for Threonine at Position 268 Uncouples Hydroxylation of Laurate. Abstract K212, p. 572. American Society for Microbiology Abstracts.

Aryl Hydrocarbon Receptor (AHR) Activation As a General Biomarker of Contamination in Aquatic Systems

C. Miller

Objectives and Goals:

The objectives of this project are the specific aims at the 1) expression of human AHR and ARNT in yeast and verification of responsiveness to activating-ligands such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD), hexachlorobenzene (HCB) and naphthoflavone (NF); 2) to determine the sensitivity and specificity of the yeast system to AHR ligands; and 3) analysis/detection of AHR agonists in biological samples.

Accomplishments This Quarter:

Milestones for this project are:

1. Expression of human AHR/ARNT in yeast
 2. Determination of sensitivity and specificity of the yeast system to various ligands
 3. Analysis/detection of AHR agonists in biological samples
- A paper describing the expression and properties of human AHR/ARNT genes in yeast is in preparation.
 - A collaboration with Dr. D. Blake (TUMC, Dept. of Ophthalmology) has been initiated in an effort to purify tryptophan for biological analysis in the yeast system being used. Whether purified tryptophan is capable of inducing AHR activation in yeast will be tested. If so, the result suggests that tryptophan or a metabolite thereof may be a ligand for AHR. The system may be used to identify the ligand.
 - Analysis of AHR/ ARNT expression in this new series of expression vectors has revealed that there may be regulation of AHR and or ARNT activity in yeast. The identification of the regulation process is under study.
 - Study of the activation AHR potential of biological samples continues. Two high school students from Ben Franklin High School will work on this as a research project.

Encapsulation and Degradation of Pollutants: Role of Zeolites and Light

V. Ramamurthy

Objectives and Goals:

1) To establish that zeolite oxidation strategy is a viable alternative to conventional oxidation of molecules. 2) To show that singlet oxygen can be generated and that can be used to oxidize organic molecules included within zeolites. 3) To establish that oxidation within zeolite can be highly selective compared to that isotropic solution.

Accomplishments This Quarter:

A supramolecular structure of olefin, a thiazine dye and oxygen has been assembled within a zeolite supercage. Excitation of the dye in this assembly results in the oxidation of olefins leading in many cases to a single hydroperoxide. Zeolite interior serves as an 'active reaction cavity' and controls the interaction between the reacting olefin and the singlet oxygen.

Although oxidation via singlet oxygen has found much synthetic application, regio and stereocontrol are still difficult issues. Oxidation with substrates containing several distinct allylic hydrogen produces several distinct products. In this system, generation of singlet oxygen entails irradiation of a thiazine dye located within the supercages of the zeolite lattice. These dyes are placed within the zeolite via a cation exchange process.

Depicted in the scheme and table below are the results of the oxidation via singlet oxygen of a series of olefins. In isotropic medium, two types of allylic hydroperoxides in a 1:1 ratio are obtained. One is a secondary (2°) while the other is a tertiary (3°) hydroperoxide. In the zeolite supercage (NaY/thionin), there is a striking selectivity for the 2° hydroperoxide.

Olefin	Conditions	2°	3°
1	Thionin / CH ₃ CN	51	49
	NaY/Thionin	72	28
2	Thionin / CH ₃ CN	40	60
	NaY/Thionin	100	—
3	Thionin / CH ₃ CN	50	50
	NaY/Thionin	85	15
4	Rose Bengal / CH ₃ CN	56	44
	NaY/Thionin	100	—
5	Rose Bengal / CH ₃ CN	52	48
	NaY/Thionin	100	—

In addition to the above acyclic olefins, a series of cyclic olefins have also been investigated. Oxidation of these olefins within the zeolite also yield unprecedented selectivity for a minor isomer. Results on these systems will also be part of the presentation.

Publications and Presentations:

Li, X. and Ramamurthy, V. Electron Transfer Reactions within Zeolites: Photooxidation of Stilbenes. *Tetrahedron Letters*. 37, 5235, 1996.

Li, X. and Ramamurthy, V. Selective Oxidation of Olefins within Organic Cation Exchanged Zeolites. *Journal American Chemical Society*. 118, 10666, 1996.

Ramamurthy, V. Photochemistry of Organic Molecules Included within Zeolites. Clarkson University. Clarkson, New York. October 24, 1996.

Ramamurthy, V. Molecular Dynamics of Chemical Reactions within Zeolites. NSF Workshop on Photoresponsive Materials. Catalina Island, CA. November 4-8, 1996.

Modeling and Assessment of Environmental Quality of Louisiana Bayous and Swamps

L. Steinberg and R. Luna

Objectives and Goals:

The goals of this initiation grant are to use available spatial and temporal environmental data on Bayou Trepagnier and Devil's Swamp to build a model describing the transport and chemical/physical/biological processes which affect contaminants in these water bodies. In the process of doing this, the most pressing environmental problems in these water bodies will be identified, and recommendations on additional sampling which would improve model predictions will be provided.

Accomplishments this Quarter:

- Hydrodynamic and geographical data for Bayou Trepagnier have been gathered from a variety of sources, including the Data Management Core and the Shell Oil Company. The data were used to segment the bayou into twenty-eight cells, each with its own characteristic hydrology and geometry. This was the basis for the creation of an input file for a hydrodynamic model (DYNHYD5) of the bayou. After running the model, the predicted water depths along a longitudinal cross-section of the Bayou were compared to measured depths, and found to match very well. The model also computes velocity of flow in the bayou; the predictions were for very slow velocity, which is what has been observed in the bayou.
- The sensitivity of the hydrodynamic model to the Manning's roughness coefficient is being investigated. It was found that by varying the coefficient value between cells, the fluctuating directional flow typically found in tidal aquatic systems can be achieved.
- The computed velocities, depths, and segment geometry from the hydrodynamic model are being used as input to the water quality model (WASP5) to predict heavy metal (lead) and organic chemical (toluene) transport and fate in the Bayou. The input data set is being assembled. Measurements of lead concentrations in the Bayou, parameters governing the sorption, diffusion, hydrolysis, and complexation of lead, and sediment transport variables are being incorporated into the data set. For toluene, parameters governing sorption, volatilization, biodegradation, and diffusion are being estimated.
- Based on the results of the hydrodynamic model, a series of parameters have been identified for more precise calibration of this model. These parameters will be presented to the Field Management Core to obtain the field data. The same approach will be used with the water quality model.

Effects of Steroid Receptor Activation in Neuroendocrine Cells of the Mammalian Hypothalamus: Mechanisms of Action of Hormonally Active Xenobiotics

J. Tasker

Objectives and Goals:

This project is designed to study the mechanisms of action of toxic chemicals in neuroendocrine cells of the mammalian brain. Some chemical contaminants exert their toxic effects on exposed organisms by binding with gonadal steroid receptors and activating steroid responsive elements within the exposed cells. Several areas of the brain concentrate gonadal steroid receptors, and exogenous steroids have been found to alter the properties of neuronal ion channels and change the responsiveness of neurons to endogenous chemical neurotransmitters. The general objective of this proposal is to determine the effects of gonadal steroids on the electrical properties of neuroendocrine cells of the hypothalamus, and to determine whether hormonally-active toxins act on hypothalamic neuroendocrine cells via steroid receptors. By combining whole-cell patch-

clamp techniques with intracellular dye injection and immunohistochemical labeling in an *in-vitro* brain slice preparation, the effects of neurally active steroids on the electrophysiology of anatomically identified hypothalamic neurons will be studied.

Accomplishments This Quarter:

- Experiments have been performed on immunohistochemically identified oxytocin- and vasopressin-secreting cells of the hypothalamic supraoptic nucleus. It was found that allopregnanolone, a progesterone metabolite, has modulatory effects on inhibitory postsynaptic currents mediated by the amino-acid neurotransmitter γ -aminobutyric acid (GABA). Allopregnanolone at a concentration of 100 nM increases the decay time constant of these currents, suggesting a modification of the open time of GABA-receptor channels. This effect has two components, one that has a short latency and is partially reversible, and the other which occurs with a relatively long latency and is irreversible. This suggests that allopregnanolone may have membrane receptor-mediated effects as well as transcriptional actions. Direct intracellular application of the steroid by including it in the patch pipette has no effect, suggesting that the observed actions may be synergistic, requiring binding to an extracellular membrane receptor. Interestingly, intracellular application of allopregnanolone, although without effects on GABA currents, occludes the modulatory effects of allopregnanolone at extracellular receptors. Investigation of the G protein dependence of this regulation is under way.
- Preliminary experiments have been undertaken using the differential display RNA fingerprinting method to determine the transcriptional effects of ovarian steroids in the hypothalamus. These experiments are still in the feasibility stage.

Publications and Presentations:

Fancsik, A. and Tasker, J. G. (1996) Modulation of GABA Currents in Hypothalamic Neurosecretory Neurons by the Neuroactive Steroid Allopregnanolone will be presented at the annual meeting of the Society for Neuroscience Abstracts in Washington, D.C., November 1996. 22:628.

Identification of Metal Accumulators Among the Flora of Contaminated Wetlands and Exploration of Their Uptake Mechanisms

J. Whitbeck

Objectives and Goals:

The goals of this project are to, first, survey and compare the structure of plant and mycorrhizal fungal communities in contaminated (Bayou Trepagnier) and relatively pristine (Stinking Bayou) swamp forest sites, then to identify plant species that accumulate heavy metals, and finally to explore the mechanisms employed by roots and their fungal symbionts in heavy metal uptake.

Accomplishments This Quarter:

- Heavy metal analysis of field-collected plant material has been completed. The following calculations have been made using this data: 1) the concentrations of several heavy metals in leaves and stems of each plant species occurring at the contaminated and pristine sites; 2) the total amount of each of these metals brought aboveground by individual species and by the plant community as a whole at each site; and 3) correlations between metal concentrations in plant tissues and total metal in the soil.
- Mean concentrations of lead, arsenic and zinc in aboveground plant tissue differ between sites, with Bayou Trepagnier lead and arsenic means are ten times greater than those in Stinking Bayou and the mean concentration of zinc in Bayou Trepagnier herbaceous material is twice that of Stinking Bayou. Two herbaceous species present at both sites, *Ptilimnium capillaceum* (Umbelliferae) and *Polygonum persicaria* (Polygonaceae), have mean leaf and stem lead concentrations 15 to 32 times greater in the Bayou Trepagnier than in the Stinking Bayou population.
- 'Trap' cultures have been set up to assess the abundance and diversity of mycorrhizal fungal species at Bayou Trepagnier. 'Trap' cultures are serial dilutions of the soil of interest mixed with biologically inert sand into which mycotrophic plants are sown. *Acer rubrum*, a common Bayou Trepagnier woody species which can form either endo- or ecto-mycorrhizal associations, and *Sorghum bicolor*, a tropical grass with high affinity for endo-mycorrhizal fungi, are the 'trap' plant species.
- Seed of the herbaceous metal accumulator, *Polygonum persicaria*, is currently being collected from both Bayou Trepagnier and unpolluted swamp forest areas for use in greenhouse studies of metal uptake mechanisms.

TECHNICAL SUPPORT ACTIVITIES

Analytical Core

W. Alworth, H. Mielke, A. Abdelghani, CIF, B. Kamath, C.J. Li, Y. Prammar, G. Wang

Objectives and Goals:

1) To assure that standardized analytical procedures are utilized for chemical analysis of samples taken in the field. 2) To provide and maintain written analytical protocols for the project. 3) To conduct sample preparation (extraction, digestion, etc.) and chemical analysis for samples collected for the project. 4) To ensure that proper laboratory safety precautions are taken. 5) To conduct appropriate QA/QC exercises to demonstrate data accuracy. 6) To coordinate with the Field Work core to ensure that samples are collected in such a manner as to meet standard holding times. 7) To coordinate with the Data Management core to ensure that analytical data is made available to the researchers on the project. 8) To provide a chemical analysis infrastructure which will be utilized to support the CBR's expanding environmental research efforts.

Accomplishments This Quarter:

- The standard analytical protocols developed last quarter were refined and revised. Copies were distributed to pertinent personnel. The protocols, which are to be used by all the core analytical facilities, have been designed to be flexible enough to allow the different investigators to study different features of environmental chemistry. The reporting, validation, and analysis of the data, however, will be enhanced by the use of the standard methods and procedures described in these protocols.
- Dr. C.J. Li began developing soil samples containing known mixtures of metals and semi-volatile organic compounds for use by the core laboratories for internal monitoring of procedures and validation of data.
- The CIF perfected methods for the analysis of the fifty target semi-volatile organic compounds in water and soil samples. Modifications are being made to the procedures for the GC/MS analysis of these semi-volatile organics from fish in order to eliminate contamination problems that have occurred.
- A goal for the fourth quarter will be to incorporate the use of Accelerated Solvent Extractor (ASE) into the protocols used by the CIF in place of the more time consuming standard soxhlet extractions presently used.
- An analytical core meeting was held September 26 to discuss the progress and to further develop the goals and aims of the core.

- Drs. Wang and Kamath are developing an extraction procedure using the ASE. Results from these extractions are being compared to those from two more conventional extractions methods: Soxhlet extraction and super critical fluid extractions. The method is being developed to replace the later two methods which take longer and consume more materials in the process than does the ASE method.
- Sediment samples (one from Bayou Trepagnier, one from Control Bayou Traverse) were obtained and used to compare the room temperature process to the more conventional hot nitric acid process. The results were similar enough to assure that the room temperature nitric acid process was an acceptable method of measurement. This safer process is now being used for all sediment sample analysis in the Xavier Lab.
- A comparison of the quantities of specific metals (lead, zinc, cadmium, manganese, copper, chromium, vanadium and arsenic) at the two field sites. The Bayou Trepagnier samples had higher metal content than those from Bayou Traverse. The largest differences were found in the quantity of lead, zinc and chromium. In the Trepagnier sediments, the highest measures 2900 mg/kg lead and 200 mg/kg zinc and 100 mg/kg chromium. In Traverse, these same metals measure 18, 75 and 25 mg/kg respectively. The data shows differences between the contaminated and control sites.
- Dr. Kale's group has taken soil and sediment samples from Bayou Trepagnier and extracted the microbiota from them. Dr. Mielke's lab conducted inorganic analyses on the environmental media from which the microbiota was extracted. Drs. Kamath and Wang analyzed the samples of the same media for organics.
- All of the participating laboratories ran proficiency testing on all elements being measured for the project to assure QA/QC.

Coordinated Instrumentation Facility

T. Lyttle, P. Burnside, W. Douglas, D. Grimm, L. Smith, III

Objectives and Goals:

- 1) To provide high quality analytical services to DOE researchers (both Tulane and Xavier)--including trace metals analysis and volatile and semi-volatile organic analysis.
- 2) To provide cost-effective equipment maintenance to DOE researchers (both Tulane and Xavier) on DOE equipment.
- 3) To provide applications support to DOE researchers (both Tulane and Xavier) for analytical projects.
- 4) To interface primarily with the Analytical core and to work closely with the Field Work and Data Management cores.

Accomplishments This Quarter:

- Inorganic laboratory successfully completed APG PET for Arsenic, Cadmium, Chromium, Cobalt, Copper, Lead, Nickel, and Zinc.

- Inorganic laboratory provided access to trace metal instruments for Dr. Flowers survey samples. Rachel Baxter, instrument operator, was responsible for initial training of students involved in Dr. Whitbeck's project, "Identification of Metal Accumulators Among the Flora of Contaminated...", and continues to provide technical support. Pierre Burnside, Inorganic laboratory manager, provided technical support for the XRF analysis.
- Inorganic laboratory provided results for the samples collected for Dr. Hartley's Biomarkers project. Rachel Baxter analyzed water and sediment samples using the ICP.
- Electronics Shop provided support to several DOE researchers for instrument repair at Xavier and Tulane Universities. Mike Badinger, instrumentation specialist, has been effecting extensive repairs in Dr. Abdelghani's lab at the TMC.
- Organic laboratory established calibration curves for the approximately 50 target compounds for semi-volatile analysis by GC/MS.
- Organic laboratory analyzed water and sediment samples from Bayou Trepagnier for Dr. Hartley's project. Sandra Braden, instrument operator, extracted the sediment samples using the Soxhlet technique and extracted the water samples using liquid-liquid extraction technique. Dr. Douglas reviewed the analytical data.
- Organic laboratory purchased and received an Accelerated Soxhlet Extractor (ASE) from Dionex. This sample preparation equipment should minimize the amount of solvent used and shorten the length of extraction times.
- Organic laboratory analyzed frog tissue for Dr. Ide and fish tissue for Dr. Bart. The samples were extracted using Soxhlet and analyzed by GC/MS.
- CIF staff worked closely with the Analytical core. Dr. Grimm, Assistant Director for Analysis, updated the methods of analysis from the various laboratories.
- CIF staff participated in the DOE retreat. Those attending included Tom Lytle, Dr. Grimm, Dr. Douglas, Rachel Baxter and Sandra Braden.

ACRONYMS

CIF - Coordinated Instrumentation Facility
 GC/MS - Gas Chromatograph/Mass Spectrometer
 ICP-AES - Inductively Coupled Plasma - Atomic Emission Spectrometer
 GFAAS - Graphite Furnace Atomic Absorption Spectrometer
 AA - Atomic Absorption (Spectrometer)
 NMR - Nuclear Magnetic Resonance (Spectrometer)
 XRF - X-ray Fluorescence (Spectrometer)
 ED-XRF - Energy Dispersive X-ray Fluorescence (Spectrometer)
 XRD - X-ray Diffractometer
 CHNO/S - Carbon, Hydrogen, Nitrogen, Oxygen and Sulfur Analyzer
 ACS - American Chemical Society

Data Management Core

C.F. Ide, S. O'Connor, S. Smith, K. Watanabe

Objectives and Goals:

The overall goal of this core group is to improve the data dissemination and data transfer among this grant's researchers. This entails the following objectives: 1) to provide a system to track samples collected in the field through analytical testing; 2) to design, develop and maintain a centralized database accessible to all of this grant's investigators; 3) to design, develop and maintain a user-friendly, efficient system for data communication and dissemination amongst the participating investigators; 4) to integrate information from external sources (state and federal agencies, industry and academia) into the database that can be useful in understanding the data currently being collected; 5) to interface the analytical core, field core into a seamless transmission of information and to interface this with the Geographic Information System (GIS) lab to provide spatial analysis of the data capabilities and mapping support to the project's researchers. 6) To provide a data management infrastructure which will be utilized to support the CBR's expanding environmental research efforts.

Accomplishments This Quarter:

- Manjula Krishnan, a graduate student, worked on the database design. The design relates the field data, the analytical data and the sample tracking data. The basic database design has been completed.
- Efforts have been concentrated on the integration of the RLIMS software into the CBR's centralized database. RLIMS is a specialized software package for managing analytical laboratory data which the EPA provided to this project at no cost.
- The prototype for data access via the internet is under development. Once the prototype is tested and refined, researchers will be able to access data generated from this project from their own computers.
- The CBR Data Management Core established a homepage. It is located at http://www.tulane.edu/~dmc_spot/. This will be a central location for various information regarding this project's research such as protocols, a link to the CBR database, etc. It is linked to the CBR's main homepage. An on-line calendar has also been established to post activities pertinent to this project. This provides an efficient mechanism to coordinate information exchange among researchers. The calendar is on the homepage.
- Data management protocols were written and distributed to investigators. A link to the protocols was put on the DMC homepage.

- Two sample delivery entry points were established: 1) a downtown campus location (J. Bennett Johnston Building) and 2) an uptown campus location (Dinwiddie Hall). The field work core will assure that all field samples will be delivered to the appropriate location. Each location has a protocol for entering sample tracking data which is transferred to the centralized database.
- Continued to work closely with the core personnel and project investigators to insure that data flow and data management needs are addressed.

Field Work Core

G. Flowers, H. Bart, S. Kale, P. Martinat

Objectives and Goals:

1) To assure that standardized collection protocols are utilized for the project's field sampling. 2) To provide and maintain written sampling protocols for all projects involving field sampling. 3) To assure that all proper permits, right-of-ways, etc. are obtained prior to field sampling and that personnel involved in sampling follow health and safety guidelines. 4) To conduct or assist in sample collection of specimens from the field sites under study. 5) To interface with the Analytical and Data Management Cores to expedite processing of samples for analysis and to insure that all field data is entered into the data management system. 6) To provide a field work infrastructure which will be utilized to support the CBR's expanding environmental research efforts.

Accomplishments This Quarter:

- Two sampling and one non-sampling trips were conducted during this quarter. Samples for analysis from Bayou Trepagnier consisted of the following: ten blue crabs, one frog, 100 fish (consisting of twelve species), forty water samples, sixteen sediment samples, three soil samples, and twelve field blanks. Samples for analysis from Bayou Traverse consisted of the following: five blue crabs, fourteen fish (consisting of seven species), twenty water samples, eight sediment samples, three soil samples, and eight field blanks. A minimal number of fish have been tagged and released to date. Ten fish have been tagged and released in Bayou Trepagnier consisting of five species and one fish has been tagged and released in Bayou Traverse.
- All samples were delivered to the CBR building where the data management core logs all samples for sample tracking. The fish were necropsied by Dr. Hartley and Dr. Martinat collected fish stomachs for analysis of contents and contamination levels. Abiotic samples and biotic tissues were then distributed to investigators and labs for analysis at both Xavier and Tulane.
- The Field Sampling Core continued water quality monitoring in Bayou Trepagnier and Bayou Traverse (control site) over the last quarter. Each month a series of stations are visited and water quality parameters (pH, temperature, dissolved oxygen, salinity, and conductivity) are recorded and entered into the project database.

Education Core

Enhancement of Environmental Education at Xavier University

S. O'Connor

Objectives and Goals:

The goal of the environmental education initiative at Xavier University is to develop a comprehensive environmental education program to produce graduates who can successfully carry out the Department of Energy's mission of environmental restoration and waste management. A major objective of the initiative is to attract minority students into environmental careers. The education project supplements research cluster projects by 1) attracting students into the environmental studies/sciences; and 2) providing academic programs relevant to the Department of Energy. The infrastructure for facilitation and coordination of programs is in place, with representatives from each school working together to build on existing strengths and resources within the four schools at the two universities.

Accomplishments This Quarter:

- Mr. LáMoyne Williams was hired as a program assistant in charge of coordinating student activities. Mr. Williams graduated from Xavier University with a degree in Biology in 1996 and replaced Bernadette Chachere.

Curriculum Development

- Two faculty mini-grant proposals were submitted in September. Dr. Reginald Gougis, Associate Professor of Psychology, submitted a proposal entitled "Environmental Psychology and Landscape Architecture." The purpose of Dr. Gougis' proposal is to request funding to enable two environmental scholars and him, as their mentor, to attend the 1996 Annual Meeting & Expo of the American Society of Landscape Architects to be held October 19 - 21 in Los Angeles, California. Ms. Stephanie Thomson, Instructor of Communications, submitted a proposal entitled "Environmental Intercollegiate Speech and Debate Program." The purpose of this project is to increase student environmental awareness through an intercollegiate debate sponsored the Cross Examination Debate Association (CEDA). Dr. Gordon Wilson, Professor of Theology, submitted a report on his infusion project entitled "Environmental Issues in Christian Perspective," which he developed in June 1996 with funding from the Center for Environmental Programs faculty mini-grant program. The syllabus and course material will be submitted this semester for review and approval by the departmental and university academic committees.
- The Center for Environmental Programs received full funding for the grant "Education for Sustainability" from the DOE through the Historically Black Colleges and Universities/Minority Institutions Consortium (HBCU/MI). The grant seeks to increase (via inspirational, informational, and technical assistance, and monetary incentive workshops) the number of faculty who will revise, infuse or develop courses to incorporate environmental concerns and issues.

- Two faculty workshops on incorporating environmental concerns into course syllabi were held at the DoubleTree Hotel, the first on April 27, 1996. Forty five faculty participants from fifteen different university departments attended. Subsequently, twenty-one (21) proposals for course infusion were received by CEP, with 20 selected for funding
- The second workshop, held on August 31, 1996, was attended by thirty-one (31) Xavier faculty and staff, and three invited speakers. Dr. Sally O'Connor opened the workshop with a short video, entitled "The Power of One," focusing on the positive social and environmental impact made by dedicated individuals in recent world history, and a review of the objectives of the project in general and the workshop in particular. Gus Von Bodungen, Assistant Secretary of the Office of Air Quality and Radiation Protection in the Louisiana Department of Environmental Quality, then gave a presentation on the development of legislation to regulate and protect environmental quality in Louisiana. Gary Groesch, Director of the Alliance for Affordable Energy, followed, speaking on the fallacy that environmental quality has to be sacrificed in the interest of maintaining or improving the economy. He cited studies forecasting the eventual effects of increasing environmental pollution, such as global warming. Dr. Geoffrey Chase, Professor/Chairman of the Department of English at Northern Arizona University, spoke about the methods used to enhance environmental literacy at his institution. Participants attended a lunch/working session, during which they compiled listings of the environmental problems they encounter on campus and proposed ways to deal with each situation. A spokesperson from each group presented the compiled listing to the entire group.
- LáMoyne Williams, CEP Program Assistant/Student Program Coordinator, gave a presentation on an updated campus recycling program being developed and proposed to the Xavier University administration. Ron Davis, Property Manager at XU Physical Plant, discussed what is being done about environmental problems at Xavier University and offered the support of the University Physical Plant for the recycling program. Dr. O'Connor closed with a discussion. Those attending agreed that, pending availability of funds, a workshop in January will be planned with the inclusion of staff and administration.

Student Development

- Ten LIFE Scholars and seventeen LIFE interns were selected by the Xavier University Environmental Scholars committee. Each has been assigned Xavier University faculty mentors to supervise them in research projects conducted during the academic year.
- The first Environmental Scholars/Interns Meeting for the 1996-97 academic year was held on September 24, 1996, in the Xavier Library Rm. 417B. Eighteen scholars/interns attended. Students submitted written updates of their research projects. Announcements were made of upcoming events, such as environmental seminars, Student Environmental Club meetings and ACT "brown bag lunches."

- The Student Environmental Club held the first Fall meeting of the semester on September 11, 1996. Clanford Johnson, Chemistry '97, welcomed and introduced the members, discussed the SEC constitution, distributed an attendance roster, and reviewed the duties of club officers. The club held an election of officers with the following results: President - Clanford Johnson (Chemistry, '97), Vice President - Raymond Brown (Biology, '97), Secretary - Christina Bolden (Biology, '01), Treasurer - LaKeisha White (Biology, '01), Historians - Angele Arthur (Biology, '01), Rudley Miland (Biology, '01), Executive Chair - Jamila Davison (Biology, '98). Other items on the agenda were club dues, and the functions of committees.
- On September 11, 1996, LaMoyne Williams, CEP Program Assistant, attended the Louisiana State Recyclers Planners Meeting in Lafayette, Louisiana, via invitation from John Rodgers, Recycling Coordinator, Louisiana Department of Environmental Quality (LADEQ), on behalf of Xavier University's newly formed recycling program and also an effort to include collegiate recyclers on the state level.
- On September 21-22, 1996, SEC Secretary, Christina Bolden, and SEC Historians, Angele Arthur and Rudley Miland, attended the 1996 Student Leadership Retreat sponsored by the XU Student Government Association (SGA) at the Airport Holidome in Kenner, Louisiana. Many topics concerning student leadership were discussed, including the importance of maintaining concentration on one's studies, handling stress, and planning community service projects.
- The 1996-97 ACT Program, sponsored by the CEP, held its first "brown bag lunch" of the semester on September 25, 1996, at the Gold-Rush Room. The ACT program is. Faculty members of the ACT program held a panel discussion offering different perspectives on this year's theme, "Environmental Sustainability: City and Country." Featured speakers were Drs. Shamsul Huda, 1996-97 Director of the ACT Program and Chairman of the History Department, Michelle Levy, Professor/Chairperson of English, Peter Martinat, Assistant Professor of Biology, Mark Thomson, Assistant Professor of Chemistry, James Lundy, Assistant Professor of Communications, and Fred Humphreys, Assistant Professor of Philosophy. The faculty also held a question and answer session on the issues raised in their discussions. The next ACT "brown-bag lunch" is scheduled for October 9, 1996, from 1 to 2 p.m.
- On September 26, 1996, the first seminar in the 1996-97 Environmental Seminar Series was held. Carlos Coffman, from the US DOE Office of Worker Safety and Health, spoke on the Industrial Hygiene Fellowship Program administered by the Oak Ridge Institute for Science and Education (ORISE). Dr. Michelle Gabriel, a Xavier University Graduate, the President of the New Jersey Chapter for the National Association for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCCHE), and currently employed as Product Development Chemist at Benjamin Moore & Co., gave a presentation entitled "After the Final Exam: Industrial Education." She described the changes in corporate culture and the new process of decision-making and team interaction, involving mission statements, performance partnerships, and the elimination of middle management. Dr. Gabriel spent the following day interviewing students for an internship position at Benjamin Moore & Co. for the summer of '97.

- Three other Environmental Seminars are scheduled for this semester. On October 10th, Lauren Nicholas, a Xavier Graduate and Program Assistant for the Deep South Center for Environmental Justice (DSCEJ), is scheduled to give a presentation on environmental justice. Willie J. McKinney, Xavier Graduate and Ph.D. candidate in Environmental Toxicology at the School of Public Health, University of North Carolina - Chapel Hill, is scheduled to give a seminar on October 17th on environmental toxicology. On November 7th, Dr. John McLachlan, Director of the Center for Bioenvironmental Research, is scheduled to give a presentation on endocrine disruption.

Enhancement of Environmental Education at Tulane University

J. W. Bennett, M. Zimmerman

Objectives and Goals:

The goal of the environmental education initiative is to develop a national center of excellence in Environmental Studies at Tulane University. Major objectives of the initiative include establishment of a Center for Bioenvironmental Research Office of Education, creation of a core environmental studies faculty, creation of a Pilot Project for Enhancing the Freshman Year, and linkage to CBR Research.

Accomplishments This Quarter:

- Ridgely Pittman, Laura Hudson and Aaron Allen were hired as student workers for the fall semester to work in the Environmental Studies Program.
- Thirty-four LAS Undergraduates students declared Environmental Studies as their major this fall.
- In August, representatives from the Green Club's Environmental Forum newsletter, the Tulane LAS Environmental Studies Program, the CBR, and the Xavier Environmental Program met to expand the Environmental Forum to include all three campuses. A proposal for increased funding was submitted to the CBR and granted. Ridgely Pittman, the current Green Club editor, in partnership with Xavier and the CBR, published their first issue of the school year in September.
- A reception for the 30 first-year students participating in the pilot Writing/Environmental Biology course, and their parents was held on August 25.
- An empowerment retreat for the 30 first-year students participating in the pilot Writing/Environmental Biology course was held on the weekend of August 30 at Fountainebleau State Park.

- Drs. Bianchi and Rothenberg, Writing/Environmental Biology team-teachers, took the class on a field trip in September to Turtle Cove, Louisiana.
- Christine Murphey, Program Coordinator, has conducted a survey of DOE-funded student research.
- Environmental Studies Honors Thesis (ENST 499/500) and Special Topics (ENST 491/492) were approved by the LAS Curriculum Committee and added to the Environmental Studies program.
- Environmental Studies Course Offerings for the Fall of 1996 include: Ecological Anthropology, General Biology and Lab, Microbiology, Diversity of Life and Lab, General Ecology and Lab, General Chemistry I and Lab, Microeconomics, Environmental Economics, Physical Geology, Environmental Ethics, Introductory Physics I, Nuclear Radiation Physics, Introduction to Political Economy, Environmental Politics, Environmental Studies Special Topics, Environmental Studies Internship.

CBR Research Training and Educational Activities

J. McLachlan, K. Davey

Objectives and Goals:

One of the goals of the CBR is to foster extensive collaborative research with such diverse disciplines as toxicology, medicine, epidemiology, cell and molecular biology, chemistry, ecology, engineering, and information sciences. Hence, the CBR sponsors activities to support research training of students and faculty and promotes education activities such as seminars and environmental courses.

Accomplishments This Quarter:

- Construction is underway on the new Research and Teaching Laboratory for Environmental Biotechnology. The lab has been designed to enhance research capabilities for clinical, basic science, and Xavier faculty members. A committee has been established to identify one Xavier faculty member and student to rotate through the Tulane-based lab starting in January, when the lab construction is completed.
- The CBR Scholars program is underway, supporting 6 students of outstanding academic achievement and scholarly promise who are pursuing a Ph.D. or who have been formally admitted to a Ph.D. program, and whose research topic is centered upon environmentally-related research. (Two of these prestigious fellowships are supported from funding through this grant.) Tulane University provides an in-kind match in the form of a one-year tuition waiver. Competition for next year's awards will start in February.

- The CBR Seminar Series brought several presentations to Tulane and Xavier this quarter, including: Julie Ostberg, University of Rochester Medical Center, Department of Immunology, *An In Vivo* Approach for Determination; Karen Gray, Ph.D., Uniformed Services University of the Health Sciences, F. Edward Hebert School of Medicine, Modulation of Lactoferrin Expression in Human Endometrial, Cervical, and Breast Cancer; Günter Vollmer, Ph.D., Institut für Molekulare Medizin, Medizinische Universität, Extracellular matrix and estrogen sensitivity: lessons learned from endometrial cancer cells.

Appendix A

Summary of Publications Resulting from the Hazardous Materials in Aquatic Environments of the Mississippi River Basin Research Project

List of Publications in Peer Reviewed Journals Resulting from Hazardous Materials in Aquatic Environments of the Mississippi River Basin Grant Funded Work

- Abdelghani, A. A., Prammar, Y. V., Mandal, T. K., Tchounwou, P. B., and Heyer, L. Levels and Toxicities of Selected Inorganic and Organic Contaminants in a Swamp Environment. *Journal of Environmental Science and Health*. 1995, B30(5): 717-731.
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- Apblett, A. W. and Georgieva, G. D. Novel Synthetic Routes for Synroc. *Ceramic Transactions*. (in press)
- Apblett, A. W., Byers, L. D. and Reinhardt, L. E. Catalytic Hydrolytic Dechlorination of Chlorocarbons. *Ceramic Transactions*. (in press)
- Barber, J. T., Sharma, H. A., Ensley, H. E., Polito, M. A. and Thomas, D. A. Detoxification of phenol by the aquatic angiosperm, *Lemna gibba*. *Chemosphere*. 1995, 31 (6): 3567 - 3574.
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