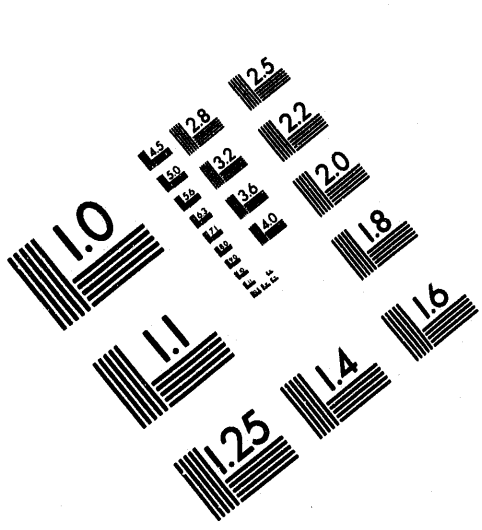

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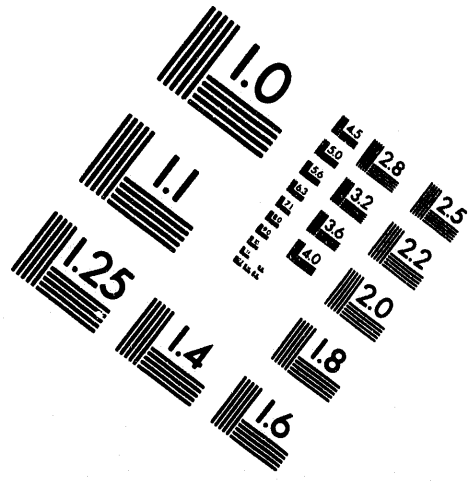

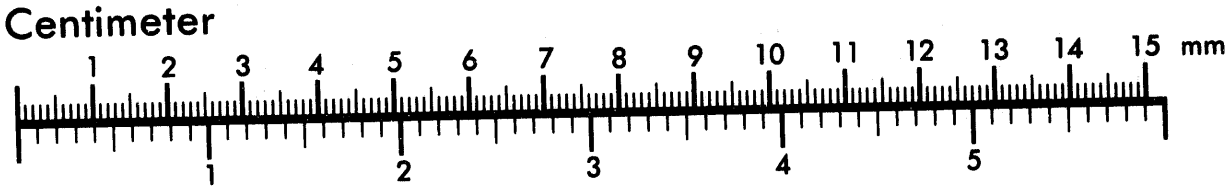

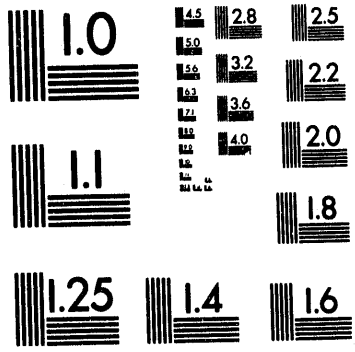

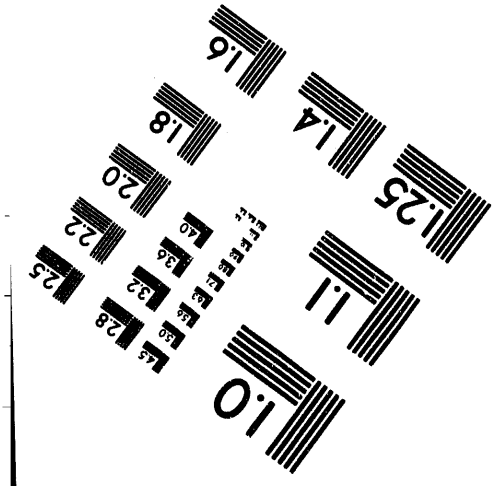

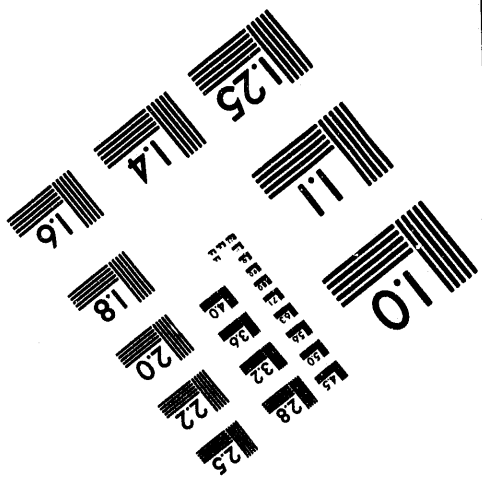

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DOE/EW/53023--T5-Exec. Summ.

Tulane/Xavier University
Center for Bioenvironmental Research

Hazardous Materials in Aquatic Environments of the Mississippi River Basin

Executive Summary of the Annual Technical Report (12/92-12/93)

Project # DE-FG01-93EW53023

Introduction

Tulane and Xavier Universities have singled out the environment as a major strategic focus for research and training for now and beyond the year 2000. In 1989, the Tulane/Xavier Center for Bioenvironmental Research (CBR) was established as the umbrella organization which coordinates environmental research at both universities. The CBR is supported by major grants from the Department of Energy, Department of Defense, National Institute of Environmental Health and Safety, National Institute of Health, National Institute of Environmental Health Sciences, and other agencies. The CBR is interdisciplinary, involving faculty and student participation from most schools and divisions at both universities and, thus, represents an integrated approach to environmental problems. Research ranging from creating new technologies for environmental clean-up to understanding the economics that drive environmental policy decisions are coordinated under the CBR auspices.

In December, 1992, the Tulane/Xavier CBR was awarded a five year grant to study pollution in the Mississippi River system. The "Hazardous Materials in Aquatic Environments of the Mississippi River Basin" project is a broad research and education program aimed at elucidating the nature and magnitude of toxic materials that contaminate aquatic environments of the Mississippi River Basin. Studies include defining the complex interactions that occur during the transport of contaminants, the actual and potential impact on ecological systems and health, and the mechanisms through which these impacts might be remediated. The Mississippi River Basin represents a model system for analyzing and solving contamination problems that are found in aquatic systems world-wide. These research and education projects are particularly relevant to the U.S. Department of Energy's programs aimed at addressing aquatic pollution problems associated with DOE National Laboratories.

First year funding supported seven collaborative cluster projects and twelve initiation projects. Over 70 faculty from Xavier University (from the School of Arts and Sciences and College of Pharmacy) and Tulane University (from the Liberal Arts and Sciences, School of Engineering, Medical School, and the School of Public Health and Tropical Medicine) participated during the first year. Additionally, more than 40 graduate and numerous undergraduate students worked on research problems associated with the project.

Study Sites

Sites in the Mississippi River basin were selected for studying how industrial contaminants enter aquatic ecosystems and how these compounds move through environmental phases and influence different species. The following areas were chosen as the major sampling sites:

- Devil's Swamp, a cypress swamp that lies northwest of Baton Rouge, is adjacent to the Mississippi River and includes a man-made lake. The swamp is polluted by a variety of surrounding industrial operations, including an abandoned hazardous waste disposal facility.
- Bayou Trepagnier (designated a "natural and scenic stream" within the Natural and Scenic River Act of 1970) serves as the receiving stream for large volumes of water used in many oil processing activities. The 3 1/2-mile bayou flows in a northeasterly direction through a cypress-tupelo swamp and was selected for study based upon its known contamination by metals, oil and grease.
- Tunica Swamp is a relatively pristine water body located approximately 20 miles up river from Devil's Swamp near St. Francisville. It is used as a control site.

Other sampling sites include: Lake Pontchartrain, Atchafalaya River, Bayou St. John and Barataria Bay.

Methods of Communication

Since this project involves numerous investigators at the two institutions, it is imperative to have well-organized modes of communication among researchers and project administrators. This is facilitated through monthly meetings that rotate among the three participating campuses (Xavier, Tulane uptown and Tulane downtown). All investigators are encouraged to attend. At each meeting, investigators from one or two selected projects present their current research findings. These presentations foster interactions among participants across projects and have resulted in the development of new, interdisciplinary research teams.

Additionally, a poster session is being planned for February, 1994. Investigators from each project will be able to present their research and answer questions from reviewers, DOE administrators, faculty, students and representatives from state and federal regulatory agencies. This venue will provide investigators with important feedback related to the next year's proposed work.

Oak Ridge National Laboratory (ORNL) is working closely with this consortia by providing research support and expertise in a variety of areas. Interactions with ORNL include visits to Oak Ridge by Tulane/Xavier researchers and administrators and student internship programs. In addition, there is close contact among project investigators and ORNL scientists. A joint seminar series has been planned in which ORNL staff will visit and give presentations on studies related to aquatic pollution that are relevant to the Tulane/Xavier project. For more details on these ORNL interactions, please see Administrative Activities.

Administrative Activities

Highlights of the past year's administrative activities are summarized below:

January
- Tulane/Xavier notified of grant award
- RFP distributed

February
- External review panel formed

March
- 35 concept papers were received
- Concept papers were peer reviewed and the panel made recommendations to: combine education PIs to form one interdisciplinary, inter-university proposal; encourage PIs working in similar areas to collaborate to enhance the research and avoid duplication of efforts
- 35 initiation project proposals and 11 collaborative cluster project proposals were received

April
- Proposals were peer reviewed
- 13 initiation projects were funded and 8 collaborative clusters were funded
- Award notification sent to PIs
- Equipment Committee formed to determine equipment needs and purchases for cluster groups
- Additional committees were formed to coordinate sampling and analyses associated with the clusters

May
- The Coordinated Instrumentation Facility (CIF) sponsored three seminars concerning environmental sample preparation techniques to educate investigators on the use of microwave digestion systems for sample preparation and the use of inductively coupled plasma and atomic absorption spectroscopy for analyses
- Relationship with Oak Ridge National Laboratories (ORNL) was established
- Student internship program developed with Oak Ridge

June
- Four students (2 from TU and 2 from XU) went to Oak Ridge for 10-week internship program

July
- Approval by DOE for equipment purchases
- Sampling started in Devil's Swamp Lake, Devil's Swamp, Bayou Trepagnier and Bayou St. John
- One professor from Xavier and one from Tulane spent 5 weeks at ORNL working with researchers and discussing future collaborations

August

- Plans were made with ORNL for an expanded summer internship program in Summer 1994
- PI meetings are planned monthly along with presentations from investigators
- Co-director toured Hanford site

September

- Drs. Carl Gehrs, Lee Shugart and Marshall Adams from ORNL visited Tulane and Xavier and presented two seminars for the project investigators
- Project administrators participated in the Office of Technology Development's (OTD) Exhibition at the Rayburn House Building and the Hart Senate Building in Washington, D.C.

October

- Project co-director participated in the Natural Resource Recovery Technology Forum sponsored by the National Environmental Waste Technology, Testing, and Evaluation Center that was held in Montana

November

- Project administrators (along with three undergraduate students that participated in the summer internship program) participated in two OTD exhibitions, one at the DOE Forrestall Building and the other at DOE Germantown
- RFP for second year funding was issued

December

- Planning began for the academic poster session to be held in February, 1994
- Review panel for second year funding was identified

Collaborative Cluster Projects

Project Title: **Biological Fate, Transport, and Ecotoxicity of Toxic and Hazardous Waste in the Mississippi River Basin**

Principal Investigator: A. Abdelghani

A report about the history and chemical concentrations of metals in soil collection from Devil's Swamp was prepared and distributed. In addition, a detailed quality assurance/quality control (QA/QC) document was prepared and distributed to all DOE Investigators. An extensive literature review was completed.

Ecology Subcluster

* Samples of fish, frogs, crayfish, bottom sediments, vegetation and water have been taken from Devil's Swamp. Early sampling was limited by equipment availability. An electrofishing boat for the project was delivered in late fall and will permit sampling of the four remaining riverine sites and the control area (Tunica Swamp).

* Cores have been taken from cypress trees in Tunica Swamp, Devil's Swamp and Bayou Trepagnier. Preliminary analysis of cypress cores from Bayou Trepagnier for heavy metals suggests cypress is a good indicator species and can be used to record past and present effects of natural and manmade events in the environment. For example, the lead levels from 1960 to 1993 are twice as high as those found in rings prior to 1960; this correlates with the time course of known lead contamination in the area.

* Most of the gastrointestinal tracts of fish from the first two collections and black crappie from the third collection have been examined . The greatest abundance of aquatic organisms was found in the stomachs of black crappies. Most organisms were entirely intact, allowing preliminary identification to family. Identification to genus or species should be possible. The most abundant were larvae and pupae of the Chironomidae and Chaoboridae.

Biomarkers Subcluster

* Comprehensive histopathological examination and documentation of approximately sixty fish from Devil's Swamp representing nine different species is in progress. Initial histopathology results indicate that pathology biomarker studies should focus on the liver, kidney, spleen, thyroid, pancreas and gills.

* Thus far, the list of significant lesions identified of particular interest with regard to exposure to chemical contaminants and disease in Devil's Swamp fish include: Channel Catfish (proliferation of alarm cells in the skin and kidney mesangiosclerosis); Yellow Bullhead Catfish (high incidence of spongiosis hepatis, telangiectasis of the liver, probable hemangioma/tumor, white pulp lesion in spleen, dilated Bowman's space in the kidney, and hyperplastic thyroid); Garfish (inflammation of muscle, melanin macrophage centers in the liver and inflammation of the pancreas); and Carp Fish (ectopic thyroid tissue in the kidney, telangiectasis of the gill, and periductal inflammation of the liver and vascular elements).

* Laboratory studies on the effects of methyl mercury on laboratory frogs show that extremely low concentrations alter gross morphological and behavioral development. Neural circuitry related to escape swimming appears altered in early swimming embryos. In adult animals the mitotic capacity of immune system cells is highly sensitive to low concentrations of methyl mercury. Laboratory studies indicate that gross morphology and behavior are dependable developmental biomarkers of exposure. Further studies will determine if neuroimmune system biochemicals such as glucocorticoids will serve as sensitive indicators of compromised immune system and nervous system function. Wild frogs caught at Devil's swamp are under comprehensive neurological and immunological evaluation with no adverse effects noted to date.

Exposure Subcluster

* The toxicity, uptake and accumulation of heavy metals by Lemna gibba (duckweed) is in progress. The acute toxicities of arsenic, cadmium, chromium, lead, thorium, and uranium have been determined with regard to vegetative reproduction of axenic cultures, grown under defined laboratory conditions. With regard to organic pollutants, statistically accurate toxicity curves for phenol, p-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,3,4,6-tetrachlorophenol, pentachlorophenol and ethylene glycol have been determined. The structures of the metabolites of the chlorophenols (chlorophenylglucosides) have also been determined.

* Studies were initiated to evaluate the physiological and biochemical effects of the heavy metal cadmium in the red swamp crayfish, Procambarus clarkii and the fiddler crab, Uca pugilator. This model approach provided the first evidence in a crustacian that cadmium exposure results in hyperglycemia in the intact crayfish. This hyperglycemia was shown to be mediated, at least partially, by the crustacean hyperglycemic hormone from the sinus glands, and neurohemal organs in the eyestalks. Exposure of crabs to cadmium resulted in decreased lactate dehydrogenase activity in the hepatopancreas but, in contrast, this enzyme activity in the abdominal muscles increased.

* Laboratory studies on the toxicity of arsenic, cadmium and mercury to crayfish and bluegill sunfish showed an order of toxicity as follows:

A.	Fish	Hg > As and Cd
B.	Crawfish	Hg > As > Cd

Mercury showed the highest toxicity (LC_{50} = 0.5 and 6.5 mg/l to fish and crayfish respectively). Arsenic and cadmium had the same toxicities to fish (LC_{50} 13 mg/l) and arsenic was twice as toxic as cadmium to crayfish (LC_{50} = 45 and 85 mg/l).

Bioassays on other species including microorganisms are planned to continue next year. Uptake, distribution, storage and depuration studies following the determination of LC50's for study chemicals will be conducted. Aquatic organisms will be exposed to concentrations based on the results of bioassays. Studies will include subchronic exposure of crayfish and bluegill sunfish to different concentrations for at least 3 months of uptake followed by 3 months of loss.

* Environmental samples including fish, frogs, crayfish, vegetation, water, soil, birds' blood, feathers, and tree cores were collected from Devil's Swamp and brought to the Environmental Health Sciences Laboratories for analysis. Most of the samples have been prepared for metal analysis (lead, mercury, arsenic, chromium, and cadmium) and organics such as hexachlorobutadiene. Preliminary results of these analysis indicate that cadmium concentrations in different fish species might pose a human health risk.

Project Title: **Assessment of Mechanisms of Metal-Induced Reproductive Toxicity in Aquatic Species as a Biomarker of Exposure**

Principal Investigator: Mary Bitner Anderson

This project is designed to identify heavy metals and organic contaminants of concern which could impact on the biota of the Louisiana wetlands by assessment of uptake and bioaccumulation of contaminants and their effects on reproductive processes as biomarkers of exposure. Field studies during the first year were focused on Bayou Trepagnier, designated a "natural and scenic stream" within the Natural and Scenic River System Act of 1970 , because of its known contamination by metals, oil and grease. Sediment and water samples, and killifish were collected from 5 different sites along the Bayou and evaluated for heavy metals and/or hydrocarbons. Heavy metal analyses of sediments demonstrated that iron and aluminum were high at all 5 sites. Lead, chromium, manganese and zinc were also present in significant amounts. The metal concentrations in the sediments were highest at site 1, located near the outflow for the Shell Complex, and site 4, located where there is a new suspect inflow stream. This latter site has a strong sulfur odor and is essentially void of aquatic life. Iron and manganese were the only metals found in high enough concentrations to be measured in the water.

Evaluation for hydrocarbons in the sediments at all 5 sites revealed the presence of saturated and unsaturated hydrocarbons over the 4 to 20 carbon chain length. While hydrocarbons and a large quantity of sulfur were present in sediments at all 5 sites, the highest concentrations were found at sites 1 and 4. Whole body hydrocarbon analyses of killifish revealed the presence of 8 different chemical compounds, characteristic of petroleum contamination.

Laboratory studies involved exposure of male and female crayfish to 150 ppb lead or 150 ppb chromium over 4 weeks or 7 weeks. There were no significant differences in body weights among any of the groups at the end of both time periods. Organ weights (hepatopancreas and gonads) expressed as percent body weight (organ index) in both the 4 and 7 week studies demonstrated no significant differences except for the ovarian index in the 4 week lead-exposed group, which was significantly increased ($P<0.01$). The ovarian indices for both the 4 and 7 week studies indicate that oocyte development for the control group, 150 ppb lead group and 150ppb chromium group are at the midvitellogenic or late vitellogenic stage of development. Thus, metal treatments at these concentrations and lengths of exposure do not appear to interfere with oocyte maturation. Testicular indices did not demonstrate any significant difference among the groups.

Determination of metal concentrations in various tissues of interest in metal-exposed crayfish is currently in progress. Lead concentrations in gills of male crayfish are not significantly different from those of the lead-exposed females. However, both are significantly different from their controls ($P<0.01$). Other tissues (hemolymph, exoskeleton, hepatopancreas, abdominal muscle, and gonads) are being analyzed for lead or chromium concentrations in both the 4 and 7 week metal-exposed groups. Histological study of the hepatopancreas after 4 weeks of metal-exposure demonstrates no obvious pathology. Histological study of the hepatopancreas after 7 weeks or exposure, as well as that of the gonads, is in progress.

Project Title: Bioremediation Of Selected Contaminants In Aquatic Environments Of The Mississippi River Basin

Principal Investigator: S. Bhattacharya

This bioremediation cluster includes several researchers from Tulane and Xavier representing departments of Biology, Cell and Molecular Biology, Chemical Engineering, Civil and Environmental Engineering, Environmental Health Sciences, and Pharmacy. Bioremediation is generally accepted as a long-term and economic treatment option. However, quantitative information on bioremediation and biosorption is required before this option can be adopted successfully. The primary goal of this on-going project is to determine the extent of natural biodegradation of hazardous organics and biosorption of hazardous organics and heavy metals by the consortia of bacteria (aerobic, anaerobic, sulfate reducing, methanotrophic, etc.), fungi (mycorrhizal, white rot, etc.), and plants. Methods to enhance the biodegradation process will be studied during the second and third years of this 3-year proposed project. The Devil's Swamp area near Baton Rouge and Bayou St. John in New Orleans have been selected as the first set of test sites. Some samples from Lake Pontchartrain, bordering New Orleans on the north, have also been analyzed.

During the first few months of Year 1, field samples were collected from selected sampling points in Devil's Swamp, Bayou St. John, and Lake Pontchartrain. It is expected that many of the contaminants found at the test site(s) are present at other sites of DOE's interest. Further, technology resulting from the proposed research involving enhanced natural biodegradation processes should be transferable to other DOE sites.

Anaerobic serum bottle studies were performed to determine the toxicity of selected organic compounds found in the samples. A report covering some of the work was sent to Dr. Tommy Phelps at Oak Ridge National Laboratory. As part of the anaerobic study, the focus was on isolating the organisms which could tolerate carbon tetrachloride. Pseudomonas cepacia appears to be the predominant organism in these samples.

Twenty species of white rot fungi and three molds have been analyzed for the ability to decolorize the polymeric dye, R-481. Decolorization of this dye is dependent on lignin-degrading enzymes useful in the biodegradation of various xenobiotics.

Site-directed mutagenesis was used to introduce amino acid substitutions into the first eight amino acid residues at the amino terminal end of the cytochrome protein, and thus far 169 mutants have been constructed, some of which have multiple amino acid substitutions. The wild type BM-3 and two mutant proteins have been purified, and a mutation of M5R was shown to alter the ability of laurate to bind to BM-3.

Samples of sediments from Orleans and Harrison Avenue bridges over Bayou St. John in New Orleans were taken on two separate dates and analyzed for heavy metals as a function of depth.

Relative to a goal of developing a rapid, sensitive, and specific method to quantitatively detect polycyclic aromatic hydrocarbons in solution, the polymerase chain reactions (PCR) for amplification of a 600 bp region of the cloned human c-myc proto-oncogene have been optimized. A linear response in the amount of PCR when starting with less than 50 ng to over 500 ng of plasmid DNA has been demonstrated.

Modeling work included sensitivity analysis of our model for substrate consumption and methane production. A model was also developed to quantify the interaction of methane-producing bacteria (MPB) and sulfate-reducing bacteria (SRB).

During the first few months, a thorough literature search was performed. The articles studied have been included in the bibliography.

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

Principal Investigator: L. Fauci

The development of effective strategies for *in situ* bioremediation depends upon understanding the detailed pore-level behavior of contaminants and microorganisms within porous media. This is due to the fact that bioavailability of microorganisms to the toxin site depends upon the local physiochemical conditions (*e.g.* pH, temperature, concentrations of dissolved gasses). First, these conditions are primary determinants of bioavailability because they influence flocculation, the propensity of microbes to aggregate and adhere to each other and the local pore structure. Increased flocculation hinders microbial migration by lessening forced convection and diffusive transport of the colloidal mixture through small pores. The local physicochemical conditions influence bioavailabilty because microbes swim preferentially by chemotaxis, the directional motion induced by variations of chemical concentrations. Thus, if concentration gradients are appropriate, microbes may more readily swim towards contaminated regions and aid in the uniform elimination of toxic waste. Once the microbes are at the contamination site, restoration will be governed by metabolic kinetics, which in turn are functions of the local physicochemical state. All of the aforementioned processes occur in a moving viscous fluid, and therefore the fluid dynamical events must be included in any realistic model. As is evident by the above description, factors controlling the local environments of microbial communities in the subsurface interstices are critical for any *in situ* remediation technology. Unfortunately, knowledge of this small-scale system has yet to be fully investigated, and is extremely complex due to the many components that govern the physicochemical and flow conditions.

During the first year of this project, the development of integrated experimental and computational models of the pore-level behavior of microorganisms was initiated. These models include the detailed analysis of convection and diffusion within the pores and the convection and chemotactic responses of swimming microorganisms to the local contaminant concentration and its spatial and temporal gradients. Additionally, these models will include microbial cohesion and adhesion to the surrounding pore structure, and the reaction kinetics of these organisms to the toxic contaminant. State-of-the-art methods of computational fluid dynamics and microscopic visualization of the adhesion/aggregation phenomena are being used.

Project Title: **Natural and Active Chemical Remediation of Toxic Metals and Radionuclides in the Aquatic Environment**

Principal Investigator: G. McPherson

Ion exchange membranes made from a polyphosphazene-based inorganic polymer are an attractive alternative to traditional electrodialysis membranes for the removal of heavy metal ions from waste water. During the first year of this project, a polymer material containing a sulfonic acid moiety on one of the phenyl groups attached to the phosphorus backbone and cross-linked with a methylene group on the other phenyl group was successfully synthesized. Preliminary analysis of this polymeric material showed that it undergoes cation-exchange behavior typical of ion-exchange membranes. The equilibrium uptake rates of Pb^{2+} and Cd^{2+} in Dupont Nafion cation exchange membranes were also determined. For salt solutions containing $Pb(NO_3)_2$ and KNO_3 or $LiNO_3$ at a total salt concentration ranging from 0.05M to 0.5M, preferential absorption of Pb^{2+} was found when the K^+/Pb^{2+} ratio in the external bulk solution was < 1.5. For the Pb-Li case, Pb^{2+} was always absorbed preferentially into Nafion, even when the Li^+/Pb^{2+} concentration ratio in the external salt solution was as high as 4:1. New microporous phosphate-based solids including cobalt-substituted solids were prepared during the first yeaf. Synthesis was carried out in 25 ml Teflon-lined bombs at temperatures between 200 and 230°C using 1,3-diaminopropane, ethylenediamine, or diphenylamine as template reagents. A violet crystalline material was prepared using H_3PO_4, $CoCO_3$, and ethylenediamine as starting materials. The material crystallizes in the space group $I4_1/a$ with a = 14.711 A, c = 17.81 A and a structural channel of 5 A. Preliminary thermal analysis indicates that this material loses water and template at 300°C and 390°C, respectively.

Natural remediation processes, adsorption and ion exchange reactions in sediments, were examined in the field and laboratory. Because the Mississippi delta has an extensive fresh-saltwater interface where the pH changes from approximately 5 (fresh water) to about 8 (salt water), several changes which impact water quality and sedimentation occur. One study underway concerns the adsorption of cadmium and lead on montmorillonite as a function of pH. Preliminary results are in accord with studies in the literature which show that the adsorption of Pb increases with pH. It is not yet clear, however, whether this increase is due to an increase in adsorption or the formation of Pb hydroxide at high pH. The role of sediment acid volatile sulfides (AVS) in limiting the concentration of heavy metals in the water column was investigated. Bottom sediments of Barataria Bay contain an average of approximately 5 μmoles/g AVS, which is considerably lower than averages for the Hudson River (12.6 μmoles/g) and Long Island Sound (15.9 μmoles/g). AVS content, unlike recoverable metal content, is not correlated with the clay content of the sample. Assuming the sediment AVS is due to the presence of monosulfides, the average scavenging capacity of bottom sediments is approximately 5 μmoles divalent heavy metals/g of sediment. Preliminary data suggests that the Barataria estuary has a limited capacity to absorb heavy metals via exchange reactions with sediment AVS. Fortunately, recoverable metal data for bottom sediments indicates heavy metal contamination is minimal in this estuary.

Project Title: Expert Geographical Information System For Assessing Hazardous Materials In Aquatic Environments

Principal Investigator: J.L. Regens

This research cluster consists of two discrete elements. Project Element #1 develops and applies GIS-based approaches to decision support for environmental restoration by delineating potential exposures and health risks at the Rocky Flats Plant and profiling contemporary and historical demographic/land use patterns at Sandia National Laboratories. Project Element #2 develops ESS software for surface water and ground water contaminants in the Mississippi River Basin.

Geographical Information System-Based Approaches to Decision Support for Environmental Restoration at Rocky Flats and Sandia

Because the DOE complex is the focus of large-scale remedial action efforts, growing concerns about the efficacy of remediation technologies, especially bioremediation of sediments and soils, underscores the need for valid, reliable data. Project Element #1 responds to the need to establish a quantitative baseline to evaluate remediation alternatives on a site-specific basis for major DOE nuclear weapons complex facilities. The development of geographic information system-based (GIS) approach to decision support for Solid Waste Management Units (SWMUs), or Individual Hazardous Substance Sites (IHSSs) in the case of Rocky Flats, within discrete Operable Units (OUs) is an essential element in meeting that need. The GIS-based approaches used in this project provide database management and analysis capability for spatial analysis, transport and fate modeling, exposure assessment, effects characterization, and statistical analysis. The project offers a way to integrate on a spatial-temporal basis available pollutant concentration, exposure, health risk, ecological attribute, and demographic/land data to characterize sites for remedial action at major DOE weapons complex facilities. Field applications are conducted at the Rocky Flats Plant and Sandia National Laboratories. The major objective of Project Element #1 is to provide spatially and temporally integrated, GIS-based approaches to decision support for environmental restoration. More specifically, this component of the project will develop and maintain a state-of-the-art GIS R&D infrastructure with a spatial analysis, modeling, and statistical analysis capability to integrate on a spatial-temporal basis pollutant concentration, exposure, health risk, ecological attribute, and demographic/land use data to characterize sites for remedial action at major DOE weapons complex or other Federal facilities.

An Expert Geographical Information System for Contaminants Fate and Transport Modeling

The research group developed a set of guidelines regarding the required capabilities and features of the GIS facility to be established on the Tulane Uptown campus. The EGIS will be designed for use by fate and transport modelers with little or no experience in GIS, database management, and computer graphics. It is a user-friendly menu-driven platform that integrates databases, statistical inference mechanisms, tools to identify modeling features for simulation, graphics and scientific visualization of input/output data, and algorithmic tools for analysis and simulation. The tools to be integrated are the contaminant transport and fate models MODFLOW, MT3D, WASP and HSPF, the GIS software ARC/INFO, and database management software ORACLE. A unified smart interface between the different software components will be developed by the research team utilizing their expertise in fate and transport modeling, soil technology, and computer science. The shell NEXPERT will be used to develop the necessary interface. The smart EGIS will be a generic platform independent of the geographical region or type of data used in the study, and could be applied to other areas with different data sets.

Education Project

Project Title: Enhancement of Environmental Education at Tulane and Xavier Universities

Principal Investigator: Sr. M. Loughlin

The environmental education initiative of Tulane University and Xavier University is to develop a comprehensive education program in an effort to produce graduates who can successfully carry out DOE's mission of environmental restoration and waste management. A major objective of the initiative is to attract minority students into environmental careers. The first year of the project focused on the development of a master plan and the creation of an institutional infrastructure that was built on existing strengths and resources of the four schools within the two universities. The master plan provided an overall blueprint of a comprehensive program that enabled funding to be obtained for individual components, and the stimulus to refine the existing environmental studies curricula and the development of new courses. The establishment of an institutional infrastructure contributed direction and stability to the initiative.

Utilizing the resources of both universities, Xavier University identified curricula that included an Environmental Studies minor, an Environmental Science track within the science disciplines, dual degree program in Environmental Engineering with Tulane's School of Engineering, and a BS/MS combined degree program with Tulane's School of Public Health and Tropical Medicine. Xavier University continued to infuse its overall curriculum with environmental topics to produce graduates in all fields who are environmentally literate.

At Tulane University, the current environmental curriculum was reviewed and a revised set of requirements for the coordinate major in environmental studies were developed for all appropriate departments in the College of Liberal Arts and Sciences. A strong science foundation will accentuate the "new" coordinate major in Environmental Studies. The Department of Civil and Environmental Engineering developed a new curriculum leading to a BS degree in Environmental Engineering. The curriculum was offered for the first time to the sophomore class of 1993 and generated much enthusiasm among women and African-American students. This program is available to Xavier students who follow the 3+2 track to obtain a BS degree from Xavier in physics and a BS in environmental engineering from Tulane. In addition, six new courses were developed at both universities for the undergraduate programs and are open to all Tulane and Xavier students.

A DOE sponsored scholarship program was introduced at Xavier University. The program employs the acronym LIFE (Living Intelligently to Foster Earthcare). Out of 35 applicants, four students were selected to receive LIFE scholarships. These scholars will be encouraged to apply for summer internships at DOE labs to further enhance their education.

Initiation Projects

Project Title: Heavy Metal Immobilization In Mineral Phases

Principal Investigator: A. Apblett

In this research, methodology has been developed by which toxic metals may be removed from aqueous solution and incorporated into ceramic wasteforms which mimic those minerals that have sequestered the hazardous metals for billions of years. In the first stage of this research thermally-unstable ligands were identified which could fulfill the role of complexing toxic metal species and allowing their precipitation or extraction into non-aqueous solvents. These were 2-ethyl-hexanoate for oxide wasteforms and ethylxanthate for sulfide wasteforms. The latter ligand was used to treat a simulated wastestream from wet lime-gypsum flue gas desulfurization plants. This led to the precipitation of the toxic heavy metals (manganese, cadmium, chromium, copper, mercury, nickel, lead, zinc and tin) while leaving the benign metals (calcium, magnesium, sodium, iron, and aluminum) in solution. Pyrolysis of the precipitate at 150°C produced a mixed metal sulfide ceramic that consisted mainly of a chalcopyrite phase. The possibility of using a long-chain carboxylic acid (2-ethylhexanoic acid as its potassium salt) to complex the metal cations associated with uranium fuel rod reprocessing waste (a simulated PUREX wastestream) so that they could be removed from aqueous solution by extraction into methylene chloride or the neat acid was studied. This approach was particularly successful at removing the actinides, lanthanides and transition metals from the wastestream. The extract was then mixed with appropriate amounts of the 2-ethylhexanoate complexes of the metals required for the ceramic matrix (Ca, Ba, Ti, Zr, Al). The solvent was then distilled (and retained for reuse) leaving behind a homogeneous mixture of the metal complexes that was amorphous to X-rays. Pyrolysis of this mixture at 800°C produced the expected Synroc phases (hollandite, $BaAl_2Ti_6O_{16}$, perovskite, $CaTiO_3$, and zirconolite, $CaZrTi_2O_7$) of the final wasteform.

Alternatively, simulated PW-4b high level radioactive waste was mixed with nitrate salts of the elements required for the Synroc matrix (except for titanium) in aqueous solution . The water was removed from the system and the residue was suspended by stirring in glacial acetic acid. An excess of acetaldehyde and the required titanium [as $Ti(OEt)_4$] was added to the mixture which was then heated at reflux for 12 hours. During that time, the nitrate salts were converted to acetates due to oxidation of the acetaldehyde by nitrate. The removal of the organic solvents from the mixture by rotary evaporator yielded a deep-red glassy solid that was completely amorphous to X-rays indicating intimate mixing of the radwaste and ceramic-matrix metal ions. This material decomposed and sintered upon heating to the usual Synroc phases without the necessity of a redox buffer since acetate provides a neutral (or slightly reducing) atmosphere upon its decomposition.

Project Title: **A Pilot Study of the Applicability of Polarography to Exposure and Bioremediation Problems in Aquatic Systems**

Principal Investigator: K. Bundy

This project has demonstrated the feasibility of using polarographic techniques to study pollution in Louisiana waters and a methodology has been developed for this purpose. Polarography is a trace level electroanalytical technique that the investigator has previously applied to biomaterials studies of surgical implants where metal levels in animal and human tissues and bovine blood have been measured. In the present project, effective methods for separation of aqueous and various particulate moieties (via filtration) and for heavy metal extraction (via acid treatment) have been validated. Water and sediment samples from Devil's Swamp have been analyzed for heavy metal concentration using differential pulse polarography. Lead has been found to be quite inhomogeneously distributed in the environment, with much higher concentrations being found in sediments and in suspended particulate form in the water as opposed to being chemically dissolved in the aqueous phase. Specifically, 3.7 ppb was found to be the dissolved concentration, while the total lead in the sediment was 19.8 ppm. The lead in the various size fractions of suspended particulate matter also appears to be quite inhomogeneously distributed. For example, in fine particulates (20-105 μm), the predominant size fraction, the lead concentration was 38.4 ppm, while in the very fine particulate (1-20 μm) it was 257 ppm. The manner in which wildlife would be exposed to this pollutant is thus quite complex and dependent on many factors. The mechanisms by which different chemical forms are dispersed in the environment and are accumulated in animals are planned to be investigated in the future via a laboratory study (in which a frog model will be used in controlled experiments to investigate internal organ uptake of heavy metals) and by collaborative research with the "Assessment of Mechanisms of Metal-Induced Reproductive Toxicity in Aquatic Species as a Biomarker of Exposure" and the "Natural and Active Remediation of Toxic and Radioactive Metals in Aquatic Environments" cluster groups.

Project Title: **An Interactive, Hypermedia Cultural Ecology Model of Risk Communication about Hazardous Waste Remediation for Scientists, Administrators and Students**

Principal Investigator: S. Duplantier

The principal investigator collected information on cultural, archaeological, historical, biological, ecological, toxicological, risk communication, and socioeconomic processes in the Mississippi River basin study area. Emphasis was placed on St. James and St. John civil parishes in the first iteration of the educational multimedia software product. The material collected was in the form of reports, printed information, photographs, maps, drawings, and video tape. The information was scanned or digitized into a Macintosh computer and authored into an interactive series of HyperCard® "stacks" which can be accessed interactively by a user of the software. The model software project can be considered for publishing in CD-ROM format once the beta testing is completed by users and stakeholders from the region.

Project Title: Bioenvironmental Analytical Support Services for DOE Clusters

Principal Investigator: W. George

The DOE Analytical Support Laboratory, an extension of the Tulane Analytical Laboratories of the Division of Toxicology, has been actively collecting field specimens, initiating field exposure experiments on catfish, as well as performing analyses of several hundred specimens by Atomic Absorption Spectrometry, Inductively Coupled Plasma Spectrometry, and GC Mass Spectrometry since October, 1993.

Analyses of 50 water, soil and biological specimens collected by field staff from specific locations in Bayou Trepagnier have been performed for the cluster project, Assessment of Metal-Induced Reproductive Toxicity in Aquatic Species Cluster. The results have been used to characterize the Bayou, identifying lead, chromium and hydrocarbons as abundant contaminants and examining the distribution of these metals and organics in the sediments and in vertebrate and invertebrate organisms from the region. Of the more than 300 crayfish tissue specimens subsequently generated in laboratory experiments on the bioaccumulation of lead and chromium, about 80% have been digested, and more than 200 are presently in the final stages of analysis. Results of completed work are presented in the final technical reports submitted by Drs. Anderson and George.

In addition, containment pens placed at various locations in Bayou Trepagnier have been stocked with catfish fingerlings in a study designed to monitor biological uptake of hydrocarbons from the sediments. Although to date, survival of the fish has been poor, efforts are continuing in this work.

The training of laboratory staff which has occurred during these initial months is evident in the increased speed and proficiency with which samples are presently being processed. Analyses rest on a strong foundation of Quality Control and Quality Assurance governing all aspects of wet chemistry and analytical procedures established by the Laboratory. While initial analyses were performed on instrumentation in the Environmental Health Sciences Department at Tulane Medical Center, laboratory personnel have also been responsible placing into service a newly purchased atomic absorption spectrometer at the Tulane Central Instrumentation Facility on the Tulane Uptown Campus.

Project Title: Evaluation of the Carcinogenic, Reproductive, and Developmental Effects of Mixtures of Contaminants on the Medaka Fish (*Oryzias latipes*)

Principal Investigator: W. Hartley

This executive summary details the initial results of a study to evaluate the toxicological effects of mixtures. The medaka fish is sensitive to a wide variety of chemicals. This study, when completed, will include cadmium in combination with other chemicals. Approximately 90% of the embryos which experienced delayed stage development in the first exposure (n=120, c=8, 4, 2, mg Cd/L) died shortly after delayed stage development was observed. The second exposure (n=25, c=1.0 gm Cd/L, 60, 30, 15 mg Cd/L) showed a slight increase in survival of embryos with delayed stage development due to aqueous cadmium exposure. Exposure of *Oryzias latipes* embryos to aqueous cadmium inhibited the proper progression of many early development parameters including improper or abnormally shaped cleavage planes, retarded development of the blastula, failure of blastula to flatten and advance into gastrulation, and improper formation of early and late gastrula. Physiological development observations revealed a decrease of heartrate during development of the exposed embryos. The trend of reduction of heartrate at the third day of development was apparent in both exposure ranges. Morphological abnormalities which were observed included improper formation of the

vascular system, improper coloration of blood, macroencephaly, irregular spinal flexure, unusual clefts in yolk sac and early cranium, improper pigmentation of trunk, failure of embryo trunk to expand to full embryonic axis, and abnormal bifurcation of embryonic axis.

Project Title: A Combined Chemical-Enzymatic Method to Remove Selected Aromatics from Aqueous Streams

Principal Investigator: V. John

Aromatics are major organic pollutants found in aqueous waste streams. Phenols, for example, are prevalent in waste streams from coal conversion processes, and are generated during coal pretreatment steps prior to combustion. Physical methods to remove such contaminants include air stripping of volatile organics and adsorption, but the techniques do not result in destruction of the contaminant compounds unless followed by catalytic oxidation. The compounds can be degraded biologically, but the extensive times for complete destruction is a detrimental factor to be considered. Chemical methods include ozonation, peroxidation, photocatalysis, and hybrid versions of these techniques.

This involved work on a novel hybrid chemical-enzymatic technique to remove aromatics from aqueous streams. The aromatic is first converted to the corresponding phenol through classical Fenton type chemistry involving catalysis by Fe(II). The phenol is subsequently polymerized through an enzymatic mechanism, using horseradish peroxidase as the oxidative enzyme. The polymer is insoluble in water and can be easily recovered. In addition, such phenolic polymers are useful products with varied applications in coatings and resins technologies. Thus, the pollutants can be eventually converted to useful products. The time scales for the proposed process are reasonable and the process is environmentally benign.

Project Title: Genetically Engineered Micro-organisms: Aromatic Hydrocarbon Biodegradation Genes From Rhodococcus.

Principal Investigator: K. Kendall

Rhodococcus sp. ATCC 19070 is capable of utilizing toluene, benzene and other aromatic compounds as sole sources of carbon and energy. This strain, and other related Rhodococci, are potentially extremely useful sources of genes that can be used to create genetically engineered microorganisms with enhanced bioremediation abilities. The aim of this initiation project is to investigate the possibility that *Rhodococcus* toluene biodegradation genes could be cloned based on homology to known genes from *Pseudomonas putida*, despite the fact that the organisms are only very distantly related.

During the first phase of this project, toluene biodegradation genes were subcloned from the pWW0 plasmid of *Pseudomonas putida* and used the various subclones as probes in Southern Blotting experiments against DNA from several species of *Rhodococcus*. It was discovered that the "*Sac*I-D" fragment of pWW0 (encoding the first part of the "lower" toluene metabolic pathway) hybridized strongly to several DNA fragments from the chromosome of *Rhodococcus* sp. ATCC19070. Three of these pieces of DNA, *Eco*RI fragments of 2.3, 2.7 and 3.5kb, were cloned into *E. coli* and the 2.3 and 2.7kb fragments subjected to sequence analysis. Although the sequence analysis is not yet complete, it has become apparent that the 2.7kb fragment encodes the majority of a previously unknown *Rhodococcus* 23s/16s/5s rRNA operon. It is unclear why

this piece of DNA would hybridize to the pWW0 *Sac*I-D fragment. However, the 2.3kb fragment appears to encode three proteins that have extensive amino acid sequence similarity to the XylX, XylY and XylZ proteins encoded by pWW0. These proteins are the three subunits of benzoate dioxygenase, a key enzyme of toluene metabolism that is required to convert benzoic acid to 1,2 dihydroxy cyclohexadiene carboxylate. In *Pseudomonas putida,xylXYZ* are the first three genes of the 14 gene "lower" or "meta cleavage" operon that is required to convert benzoate to pyruvate and acetate, simple compounds that can be utilized by basic cellular metabolism. Although the actual DNA homology is much weaker, the *Rhodococcus xylXYZ* sequence suggests that the gene organization is likely to be similar to pWW0. Thus it should be possible to use the cloned 2.3kb *Eco*RI fragment as a hybridization probe to isolate the remainder of the benzoate metabolism genes.

In summary, the major goal of this initiation project - to isolate genes required for toluene metabolism in *Rhodococcus* sp. ATCC 19070 was achieved. The cloned *xylXYZ* genes can now be used to isolate the remainder of the toluene biodegradation pathway as a first step towards creating genetically engineered microorganisms with enhanced bioremediation abilities.

Project Title: Laser Ablation/Ionization Studies Related to the Removal of Nuclear Materials from Metal Surfaces

Principal Investigator: B. Koplitz

This project uses ablation and ionization lasers to study the ablation of contaminated metal surfaces. Experimentally, laser ablation of the metal of interest is carried out physically below an electric field using either 308, 248, or 193 nm radiation. One of the plates forming the electric field is actually a wire mesh that allows ions to pass. A second laser beam (193 nm; 6.4 eV) is positioned to actively ionize neutral species that are formed during the ablation pulse and ejected upward from the surface. Ionized species are directed down the flight tube of a time-of-flight mass spectrometer (TOFMS) so that their nature can be identified. The laser ablation and ionization properties of various metals under two general sets of conditions can be studied: (1) ablation laser alone with pulsed electric field and (2) ablation *and* ionization lasers with pulsed electric field. With bare Ni or Fe as the target surfaces, the photon energy of the ionization laser (6.4 eV) is below the ionization potential of the individual atoms, so only larger clusters (i.e. those whose ionization potential approaches that of the work function of the metal) can be identified. However, monitoring cluster distributions as a function of laser fluence will lend insight into the laser ablation process.

Project Title: Asymmetric PVDF Pervaporation Membranes for the Removal of Organic Contaminants from Waste Water

Principal Investigator: P. Pintauro

Asymmetric polyvinylidene fluoride (PVDF) pervaporation membranes were investigated for organic/water separations. Due to the hydrophobic nature and chemical stability of PVDF polymer, these membranes are ideally suited for the selective removal of organics (e.g., benzene, toluene, xylene, chloroform, ethyl acetate, and alcohol) from dilute aqueous solutions via a pervaporation separation process. Pervaporation experiments were performed with wet cast PVDF membranes and dilute aqueous feed solutions of *o*-dichlorobenzene (70 ppm) and

styrene (100 ppm). Both organics have boiling points greater than that of water and trace quantities of these compounds cannot be removed from water by air stripping. A flat sheet membrane pervaporation apparatus was used to collect flux and separation factor data where the total membrane area exposed to the feed solution was 225 cm^2. A vacuum was applied on the downstream side of the membrane to generate permeate fluxes. The effects of feed temperature (30, 45, and 60°C) and downstream pressure (0.03, 0.05, and 0.08 atm.) on the organic separation factor and transmembrane organic flux were determined (the organic separation factor is defined as the ratio of the wt% organic to wt% water in the permeate divided by the ratio of the wt% organic to wt% water in the feed solution). Although the PVDF membranes exhibit an asymmetric microstructure (as determined by scanning electron microscopy), we found identical organic fluxes and separation factors when either the dense or porous sides of the PVDF film faced the feed solution (so long as the linear velocity of the feed fluid over the membrane surface was > 14 cm/s in order to eliminate concentration polarization). The PVDF membranes work very well with both high organic separation factors (80-1254 for *o*-dichlorobenzene and 808-3921 for styrene) and high transmembrane organic fluxes (0.46-2.04 g/m^2-h for *o*-dichlorobenzene and 0.42 to 6.7 g/m^2-h for styrene). For comparison purposes, the separation factor for a 20 ppm *o*-dichlorobenzene in water feed solution using a polyether block amide (PEBA) pervaporation membrane was found to 1020 and the separation factor for a 35 ppm styrene in water feed (with a PEBA membrane) was 741.

Project Title: **Initiation of Research Collaboration Between the Tulane/Xavier CBR and the Institute of Radioecological Problems in Minsk, Belarus**

Principal Investigator: **S. Ramer**

This grant was designed to lay the foundations for a program of collaborative research between the Tulane/Xavier Center for Bioenvironmental Research (CBR), which is studying Hazardous Material in Aquatic Environments of the Mississippi River Basin, and the Institute of Radioecological Problems (IREP) of the Belarus Academy of Sciences in Minsk. Most of the activities of the IREP today are directly related to the Chernobyl catastrophe and its impact upon Belarus. In order to determine the most fruitful areas for scientific collaboration, investigators visited the IREP. The goal of the visit was to define the general parameters of a specific collaborative project. In the course of the visit, it was decided that IREP's mathematical and computer modeling laboratory, whose members have developed a variety of computer models concerning the fate and transfer of radionuclides in Belarus, would provide the best research match with this program. Mathematical modelers at Tulane and Xavier were sought to participate. Dr. Efstathios E. Michaelides of the Department of Mechanical Engineering at Tulane, and other engineers will be collaborating to develop a proposal which will seek funding to further this research interaction. The topic of the proposal will focus upon the fate and transport of radionuclides in several river basins of southeastern Belarus and also the fate and transport of radionuclides resulting from forest fires. During the fall semester, Mr. Oleg Pimenov, a graduate studying at the IREP, was invited to enter Tulane's graduate program in Mechanical Engineering. Tulane provided Mr. Pimenov with a tuition waiver and graduate stipend for the period January - September, 1994.

Project Title: **Risk, Stress, and Restructuring in the U.S. Petrochemical Industry: A Case Study from Louisiana**

Principal Investigator: J. T. Roberts

In communities surrounding hazardous facilities and disaster areas, it has been documented that there is a relationship between the perception of toxic exposures and mental health outcomes. This project examines whether this relationship also occurs in the workplace. Namely, do employees experience stress effects based on their perceptions of exposure to health risks on the job? A questionnaire was constructed and data collection was completed at a chemical facility near Baton Rouge, Louisiana where 238 of 356 surveys distributed were returned, for a response rate of about 64 percent. This study documents that the relationship between perception of exposure and workers' off-the-job psychological functioning is contingent on two factors. First, workers who perceive exposures as threats and worry about them are more likely to report higher levels of anxiety and anomie (alienation from other people and society). Second, depression was not directly linked to exposures and worry, indicating that there may be a more complex relationship between exposures and depression. Future research will examine whether feelings of uncertainty of the effects of exposures or helplessness to prevent them may be critical variables in explaining depression. These results were presented at the Eastern Sociological Society annual meetings in Baltimore March 17-20, 1994.

A secondary data analysis was conducted on worker stress and perceived hazards using data from the International Social Survey Project (ISSP), including representative samples of eleven nations in North America and Europe, plus Israel. The relationship between "unhealthy" and "stressful" work held up in eight of eleven countries. The resulting article has been accepted for presentation at the American Sociological Association (ASA) for its annual conference upcoming in August, 1994. Finally, in October, 1993 an application was submitted for two years of funding to complete this project to the National Institute for Occupational Safety and Health through their small grants program.

Project Title: **Selective Complexation Of The Uranyl Ion Using Modified Polymeric Supports**

Principal Investigator: D. M. Roundhill

The compound *N*, *N'*-dimethylaminomethylenebis (phosphonic acid) and aminomethylenebis (phosphonic acid) have been synthesized, and their aqueous solution chemistry with the $UO^2{}_2{}^+$ ion studied. The stability constants have been obtained by potentiometric methods for both the 1 : 1 (ML^{2-}) and the 1 : 2 ($ML^6{}_2{}^-$) complexes. These complexes have high stability constants (ß) ranging from values of 24.8 (0.6) for ML^{2-} with MAMDP, to 32.4 (0.5) for $ML^6{}_2{}^-$, also with MAMDP. The complexes are stable over a wide range of pH values, thereby making the ligands suitable for environmental applications. Techniques have been developed to chemically attach ligands of this type to polymeric supports such as polyethyleneimine.

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