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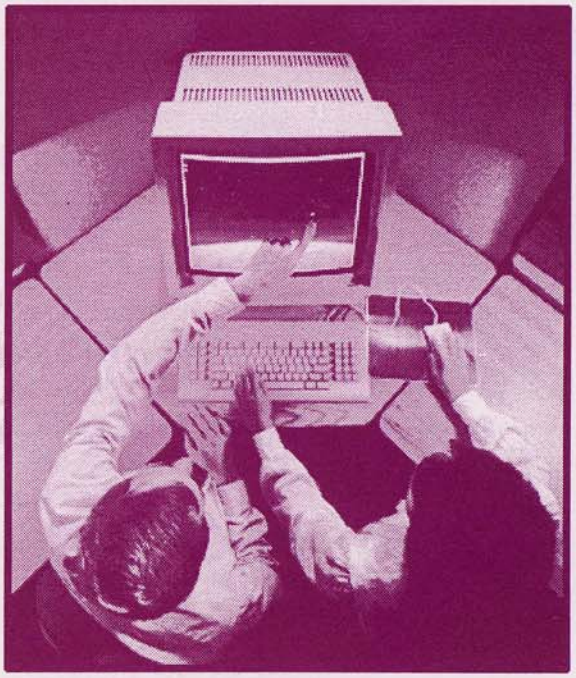
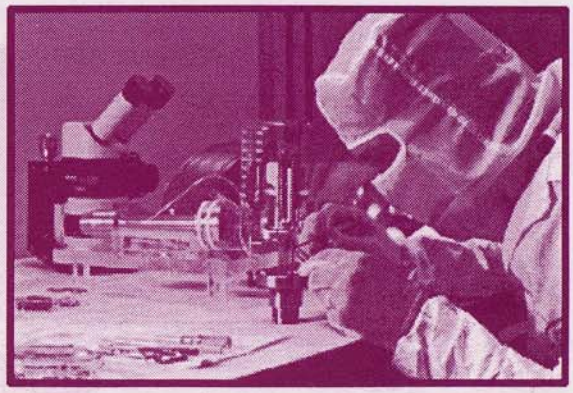
EPA

# SCIENCE TO ACHIEVE RESULTS (STAR) GRADUATE FELLOWSHIP PROGRAM

## Technical Conference

**June 16-17,1997**
***Research Triangle Park, North Carolina***





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**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**
NATIONAL CENTER FOR ENVIRONMENTAL RESEARCH
AND QUALITY ASSURANCE
WASHINGTON, DC 20460

OFFICE OF
RESEARCH AND DEVELOPMENT

Dear STAR Graduate Fellows:

Welcome to the first U.S. Environmental Protection Agency (EPA) Annual STAR Technical Conference for graduate fellows. We are delighted to be able to provide this opportunity for you to learn more about the work of EPA and of your fellow STAR recipients, and to provide the Agency's scientists and engineers with a glimpse of the talented and motivated environmental researchers of tomorrow.

EPA is deeply committed to the goal of improving the technical basis for environmental decision-making. Toward that end, we have taken several dramatic steps in recent years including the initiation of large-scale improvements in the research infrastructure of the Agency, a re-organization of the Office of Research and Development to enable technical staff to devote their time more fully to the advancement of science, a renewed program of technical outreach and information sharing, and the establishment of our flagship -- the STAR program.

STAR stands for Science to Achieve Results and it represents EPA's recognition that both immediate and future improvement of the environment depend upon expanding our current academic research capabilities, improving the exchange of technical information, and inspiring and nurturing the next generation of environmental scientists, engineers, and policy-makers. The STAR program consists of four integrated programs aimed at achieving these goals. These are the:

- ***STAR Exploratory Research Grants Program*** -- which currently provides roughly $20 million annually to support investigator-initiated research at US universities.

- ***STAR Focused Request for Applications*** -- $70 million is devoted to the support of research in response to targeted requests for applications in specific areas of current and emerging environmental interest.

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- ***STAR Environmental Research Centers Program*** -- which provides a long-term, stable funding base for collaborative and interdisciplinary research on complex or emerging environmental problems. Currently, there are four such competitively-established university-based centers which each receive $1 million annually.

- ***STAR Graduate Fellowship Program*** -- We are entering our third year in this exciting and rewarding program and by September, 1997, we hope to be providing support to 300 students in universities throughout the country.

The promise of a sustainable environment depends upon you and the other dedicated young minds of your generation. You are our legacy and our hope for a restored and vibrant world. My colleagues and I at EPA are proud to be a part of that legacy.

Congratulations on your achievements to-date, best wishes for continued success, and enjoy your stay at EPA.

Sincerely,

Peter W. Preuss

Peter W. Preuss, Ph.D.
Director, National Center for Environmental Research and Quality Assurance

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*ABSTRACTS*

# 1997 Science To Achieve Results (STAR) Graduate Fellowship Technical Conference

June 16 - 17, 1997
Research Triangle Park, North Carolina

## INTRODUCTION

The U.S. Environmental Protection Agency (EPA) initiated the Graduate Education Fellowships Program in 1995 as part of the Office of Research and Development's **Science To Achieve Results** (STAR) program. The purpose of the fellowship program is to encourage promising students to pursue advanced degrees and careers in environmentally related fields. Both the public and private sectors will need a steady stream of well-trained environmental specialists if our society is to meet future environmental challenges. Through this program, EPA continues to provide leadership in the nation's environmental science, research, education, assessment, restoration, and preservation efforts.

These fellowships are intended to help defray costs associated with advanced study in academic disciplines relating to environmental management, including physical, biological, and social sciences, mathematics and computer science, engineering, and environmental law. Students in Masters programs may receive support for up to two years. Doctoral students may be supported for a maximum of three years. The fellowship program provides up to $34,000 per year of support, depending on the number of months covered by the student's enrollment. This amount includes a $17,000 annual stipend, $5,000 for authorized expenses paid to the student, and up to $12,000 for tuition and fees paid directly to the institution. EPA's goal is to have 300 students in the program by the fall of 1997 and to award approximately 100 new fellowships each year to students at universities across the country.

This document contains 133 abstracts describing some of the research projects in which these talented students are currently engaged. Each abstract includes the project goal, rationale for conducting the research, experimental approach, and status of the research to date. The projects reflect a diversity of research fields, including ecology, geophysics, toxicology, public policy, economics, and engineering.

Information for contacting scientists and engineers within the Office of Research and Development's laboratories and centers located in Research Triangle Park, North Carolina, is provided at the end of this document.

# *Office of Research and Development*
# *Contacts in*
# *Research Triangle Park, NC*

# DEVELOPMENT OF REDOX ZONES IN CONTAMINATED AQUIFERS: IMPLICATIONS OF THE REACTION NETWORK ON SOLUTE TRANSPORT MODELING

**Robert H. Abrams**
Department of Geological & Environmental Sciences
Stanford University

**Goal:** Redox conditions influence the mobility and toxicity of metal contaminants and the ability to bioremediate organic contaminants. The goal of this project is to establish a physics and chemistry based simulation protocol to quantitatively estimate the fate and transport of contaminants that are influenced or controlled by different redox conditions.

**Rationale:** Groundwater contamination sites are often characterized by a sequence of redox zones. Assessment of many subsurface contaminants depends upon quantitative characterization of the development and fate of redox zones. The ability to simulate these zones will greatly enhance predictive modeling of the fate of many inorganic and organic contaminants at hazardous waste sites.

**Approach:** The approach for model development and testing in this study is: (1) development of general coupled subsurface fluid flow, solute transport, and geochemical models for redox zones; (2) sensitivity analysis for the chemical components/reactions and hydrologic variables essential to the development of a robust model of field-scale phenomena; (3) application of the coupled models to the U.S. Geological Survey's extensively studied research site on Cape Cod, MA; and (4) quantitative model performance evaluation for the case study based on both statistical and graphical criteria.

Microbial degradation of dissolved organic carbon coupled to the reduction of oxygen, nitrate, manganese oxides, and ferric hydroxides forms the foundation of redox zones. The ability of microbes to catalyze these reactions is limited by thermodynamic considerations. To use thermodynamics in geochemical simulations, particularly in coupled solute transport/geochemical simulations, highly nonlinear and ill-conditioned systems of equations must be solved. The successful solution of these systems of equations in the context of transient solute transport simulations requires the development of a new simulation protocol. A rational method was developed that focuses on the dominant reactions in any given geochemical zone, effectively eliminates insignificant reactions, and greatly improves numerical stability.

Since redox reactions are notorious for being slow, rate limitations are placed on the reactions. The reaction network is constrained by thermodynamics and kinetics, resulting in a theoretically-based model capable of simulating microbial processes and the effect they have on the chemical environment of the aquifer. The reaction network is coupled first to a one-dimensional solute transport model for qualitative model performance evaluation, and subsequently to a two-dimensional model of subsurface fluid flow and solute transport for the Cape Code site. The data sets from this site are used to quantitatively evaluate model performance and guide model improvements.

**Status:** A manuscript detailing the procedure that was developed to solve the reaction network under thermodynamic constraints has been prepared and is ready for submission to *Water Resources Research.* I am currently working on a computer code that will impose kinetic constraints on the thermodynamic reaction network and have performed preliminary, one-dimensional solute transport simulations that include a partial reaction network subject to both types of constraints. This critical phase will be followed by development of a two-dimensional saturated/unsaturated fluid flow and solute transport model for the Cape Cod site, to which the reaction network will be coupled. Concurrently, I will be analyzing field data for the period 1978 through 1997 which will provide the foundation for evaluating the coupled model of the Cape Cod site. I expect that this research project will be completed within 12-14 months.

# DETERMINATION OF URBAN-SCALE EMISSIONS USING INVERSE AIR POLLUTION MODELING

**Gary Adamkiewicz**
Department of Chemical Engineering
Massachusetts Institute of Technology

**Goal:** To use inverse modeling techniques as a means of estimating the spatial and temporal structure of emissions that contribute to air pollution in cities such as Los Angeles.

**Rationale:** Urban-scale air pollution continues to be a problem in metropolitan areas worldwide despite large investments in control strategies. Emissions inventories used in airshed modeling and control strategy design have been widely underestimated. Tunnel studies suggest that actual emissions from mobile sources may be three to four times higher than inventory values. Inaccuracies in base-case emissions have led to inefficient control strategies. Since a detailed reassessment of all emissions sources is an expensive and difficult task, inverse modeling can be applied to this problem.

**Approach:** This work presents a new approach to solving the inverse problem. One method of reducing the number of variables in a field is to represent the field by an orthogonal expansion, such that the new design variables are the expansion coefficients. Such an expansion should retain as much of the field structure as possible and require the fewest number of terms possible. These requirements suggest the use of the Karhunen-Loeve (KL) procedure with empirical eigenfunctions. KL expansions have been used in many disciplines to reduce the dimensionality of the problem when there is a strong correlation between points in the field. Emissions fields can thus be represented by a KL expansion as an optimal approximation. An optimization algorithm can be applied that retains the principal structure of the fields as prior information, searching for the "true" emissions fields.

**Status:** The method has been applied to determining the spatial and temporal structure of reactive organic gas (ROG) and carbon monoxide (CO) emissions in the Los Angeles basin. The resulting emissions estimates are consistent with published studies based on alternative methodologies. These results are indicative of the errors that exist in emissions inventories nationwide. In a region where the structure of the emissions fields can be inferred from known data, this procedure can be used to obtain improved emissions estimates. The method will be applied next to specific sources within the LA basin and Mexico City. Several issues concerning the optimization will be addressed in future work, including: (i) evaluation of the errors in the resulting fields; (ii) sensitivity of optimal fields to the amount of observational data used; and (iii) the "uniqueness" of optimal fields.

# THE IMPACT OF ULTRAVIOLET RADIATION ON ESTUARINE CRAB LARVAE

**Jean R. Anastasia**
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State University of New York at Stony Brook

**Goal:** This research was designed to answer the following questions: Are estuarine crab larvae that remain in near surface waters affected by ultraviolet radiation (UV)? Do these larvae suffer increased mortality due to photodamage or do they have adaptations, such as pigmentation, to counter their increased exposure to UV?

**Rationale:** Stratospheric ozone depletion has resulted in an increase in the amount of UV reaching the Earth's surface. Many estuarine larvae must frequent surface waters to complete their horizontal migrations, and the effect of UV on these organisms is relatively unknown.

**Approach:**

1. Effect of UV on larval survival: Larvae were placed in trays that were either covered with plexiglass to block UV or open to full sunlight. These trays were floated in the field and larval survival was monitored daily. Light intensity was also measured daily with a LiCor meter.

2. The effect of UV on chromatophore dispersion: If chromatophores can protect larvae from UV, then larvae may expand their chromatophores in direct response to UV. The dispersion index of a chromatophore was scored on each larva. Larvae then were placed in small culture dishes either exposed to full sunlight or covered with plexiglass to block UV. After 1, 5, or 10 min of exposure, larval chromatophore dispersion indices were again scored. The degree of dispersion was quantified using a five point scale.

3. Endogenous rhythms of chromatophore dispersion: If UV is an important factor and chromatophores protect larvae from UV, larvae are expected to expand their chromatophores as they near the surface and condense their chromatophores as they move lower in the water column. The results of this experiment were correlated with larval vertical migrations patterns to see if this hypothesis is supported. Larvae were kept in small culture dishes in total darkness in the lab. Every two hours, the dispersion indices of three larval chromatophores were scored on each of ten larvae.

4. Endogenous rhythms of vertical migrations: Endogenous vertical migrations of larvae were documented by placing larvae in tall (180 cm) tubes in the lab in darkness and counting the number of larvae in each 10 cm section of the tube every two hours with the aid of dim red light. These data were correlated with rhythms of chromatophore dispersion and will later be correlated with vertical migrations in the field with and without UV.

**Status:** The experiments were all performed using fiddler crab *(Uca pugilator)* larvae and I hope to repeat them with blue crab *(Callinectes sapidus)* larvae. I am preparing to document vertical migrations in the field when larvae are exposed to or shielded from UV. I am currently working on a method to quantify larval pigmentation to see if the amount of pigment correlates with larval survival when exposed to UV. I hope to complete this research by the summer of 1998.

# ASSESSING IMPACTS OF COMBINED STRESSES ON RIVERINE ECOSYSTEMS

**Jamie Anderson**
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University of California, Davis

**Goal:** To aid in development of strategies for management of sustainable riverine ecosystems, this research seeks to provide a methodology to quantify temporal and spatial changes in ecosystem health induced by multiple stresses. An initial application of this methodology will be in the analysis of the ecosystem that supports endangered winter-run chinook salmon in the Sacramento River in California.

**Rationale:** Riverine systems that once supported healthy communities of aquatic species have been developed to meet multiple water use objectives. The effects of such developmental stresses have been especially notable in species that are sensitive to shifts in water quantity and quality, such as Pacific salmonids. Additional stresses may be imposed on the riverine ecosystem by natural sources. State and federal agencies are spending millions of dollars in efforts to protect endangered species such as winter-run chinook salmon affected by multiple stresses. Improved understanding of riverine ecosystem behavior under stress would aid in finding practical methods of preservation for endangered aquatic species and habitat.

**Approach:** This research focuses on development of analytical techniques to quantify changes in ecosystem health induced by combined stresses. Important processes that will be quantified include mass and energy exchange among system components; advective and diffusive transport; and lethal and sublethal toxicological effects on selected sensitive species.

In order to analyze spatial and temporal variations in the physical, chemical, and biological environment, a dynamic analysis approach has been selected. A mechanistic ecosystem response model is being developed that integrates three sub-models of the hydrodynamic, water quality, and biological components of the riverine system. Existing hydrodynamic and water quality models are being applied to describe the physical and chemical characteristics of the system. A biological model is being developed that includes stress/response relationships of organisms to selected environmental factors. The ecosystem response model will be tested utilizing available field and laboratory data. Management alternatives that modify the stresses experienced by aquatic species will be developed and analyzed.

**Status:** The ecosystem that supports the endangered winter-run chinook salmon in the Sacramento River in California has been selected for initial development of this methodology. Hydrodynamic and water quality models have been applied to the Sacramento River system. These models are currently being calibrated to field data. A particle tracking model has been selected as the foundation for the ecological model. The particles will represent individual or sub-populations of juvenile chinook salmon. Survival, growth, and migration relationships that are functions of selected physical and chemical parameters are being developed.

# THE EFFECT OF CHLOROTHALONIL ON IMMUNE SYSTEM FUNCTION IN FISH AND OYSTERS: MECHANISMS OF TOXICITY

**Caroline Baier-Anderson**
Program in Toxicology, University of Maryland at Baltimore and
Chesapeake Biological Laboratory

**Goal:** The purpose of this study is to characterize the effects of the fungicide chlorothalonil (TCIN) on the phagocytic cells of the immune system of the striped bass, *Morone saxatilus,* and the oyster, *Crassostrea virginica,* and to identify the mechanism(s) of toxicity by probing the pathways of cellular activation.

**Rationale:** There is increasing evidence that exposure to chlorinated pesticides can suppress immune system function in several aquatic species. Chlorothalonil (TCIN) is a fungicide that is commonly found in creeks and estuaries, including the Chesapeake Bay ecosystem. It is therefore of interest to examine the effects of TCIN on these economically important species, since immunosuppression can result in devastating losses due to disease. By probing the mechanisms of toxicity using *in vitro* techniques, we may identify more sensitive biomarkers with trans-species applications, and may aid the development of safer alternatives to pesticides in current use.

**Approach:** Macrophage-enriched cell populations from striped bass were obtained by removing the anterior kidney, homogenizing the tissue to obtain a single cell suspension, and separating the cells by density-gradient centrifugation. Oyster hemocytes were collected by notching the valves and withdrawing hemolymph. The cells from individual organisms were then exposed to a range of TCIN (10-500 ppb) for 20 hours, after which they were washed and resuspended in clean buffer. To characterize the effects of TCIN on phagocyte function, phagocytic capacity was measured by introducing FITC-conjugated yeast to the cells, allowing for particle uptake, and then applying quencher and measuring fluorescence (phagocytosed particles will continue to fluoresce). Reactive oxygen species (ROS) production was measured by luminol-augmented chemiluminescence using either phorbol myristate acetate or zymosan as the immunostimulant. To rule out non-specific toxicity, cell viability was assessed with the trypan blue exclusion assay. Additionally, the thiobarbituric acid reactive substances (TBARS) assay will be used to determine if TCIN triggers lipid peroxidation. Since the toxicity of TCIN has been linked to loss of glutathione, cells were incubated with buthionine sulfoximine (BSO—an inhibitor of reduced glutathione [GSH]) with and without maleic acid diethyl ether (DEM—a mitochondrial GSH inhibitor) and assayed for phagocytosis and ROS production.

**Status:** To date, TCIN has been shown to decrease ROS production in both species but does not interfere with the phagocytic capacity. Cell viability is not a factor in the observed decrease. Loss of cytosolic GSH does not appear to be the cause of decreased ROS production. These results lend weight to the hypothesis that TCIN interferes with the activation of NADPH oxidase. This clearly merits further study to determine the effects of TCIN on the efficacy of protein kinase C activation (a prerequisite to NADPH oxidase assembly) and on the assemblage of the NADPH oxidase enzyme complex, using immunoblotting techniques.

# THE EFFECT OF LEAD EXPOSURE ON DOPAMINE PHARMACOLOGY AND ATTENTIONAL FUNCTION

**Lorna Bayer**
Department of Psychology
Cornell University

**Goal**: The goal of this project is to assess the involvement of dopamine neuropharmacology in the cognitive/attentional dysfunction induced by exposure to low-level lead contamination.

**Rationale**: Environmental lead contamination poses a serious health risk, particularly to the very young. The blood Pb (BPb) deemed acceptable by the CDC was recently lowered from 25 to 10 g/dl, suggesting that even "background" levels of lead contamination can cause significant neurobehavioral deficits. Evidence for enduring cognitive dysfunction suggests that Pb exposure may cause enduring alterations in neuropharmacology and/or neurochemistry. Identification of these underlying chemical alterations is an important first step in the development of therapeutic intervention strategies.

The dopaminergic neurotransmitter system seems a particularly good candidate for the basis of the Pb-induced cognitive impairments. Exposure to low levels of Pb is associated with selective impairments of performance on tests associated with the functioning of the prefrontal/striatal DA system. The studies described below use behavioral endpoints to provide a sensitive method of assessing the link between Pb-induced cognitive deficits and underlying Pb-induced changes in the DA system.

**Approach**: This series of investigations assesses the role of the DA system in Pb-induced attentional deficits using both behavioral pharmacology and receptor binding approaches. Rats exposed to Pb and control rats are tested on tasks assessing attention and distractibility, processes dependent on an intact prefrontal DA system and particularly on Dl dopamine receptor function.

In the behavioral pharmacology component of this investigation, rats are administered a series of doses of the selective full Dl agonist SKF 81297 and/or the selective Dl antagonist SCH23390 immediately prior to testing on the attentional task. Dose-response (D-R) curves are generated and examined to determine if D-R function has been shifted in the Pb-exposed animals relative to the controls. Such a shift would indicate that alterations in Dl receptor sensitivity contribute to observed performance differences on these tasks. Four separate studies compare the effects of these compounds on control rats with effects on rats exposed to lead either during early development or chronically post-weaning (a model of human occupational lead exposure). An additional experiment assesses the effect of idazoxan, an alpha-2 adrenergic antagonist, on attentional task performance in rats exposed to lead chronically post-weaning and in controls, to test the role of norepinephrine in lead-induced attentional alterations.

In the receptor binding study, a separate group of drug-naive rats are tested on the same attentional task. Following testing, animals are sacrificed and radioligand binding assays are performed to determine if differences exist between rats exposed to lead during early development and control rats in the Bmax or KD of 3H-SCH23390 binding to Dl receptors in prefrontal cortex, striatum, or nucleus accumbens. The correlation of the observed Bmax and KD values with cognitive task performance and with BPb level is determined. If altered cognitive performance in Pb-exposed animals is due to changes in sensitivity of Dl receptors, alterations in radioligand-receptor binding should correlate with BPb levels and with level of cognitive task performance.

**Status**: The norepinephrine behavioral pharmacology study has been completed. Testing and data collection are complete for the DA behavioral pharmacology experiments. Behavioral testing and binding assays are complete for the receptor binding study. Data analysis is underway for both components. In-depth analyses of response patterns will be completed for all behavioral data to allow correlation of deficits in specific cognitive processes with pharmacological measures. This research should be complete by August, 1998.

# PHOSPHORUS CYCLING IN THE GULF OF MAINE: A MULTI-TRACER APPROACH

**Claudia R. Benitez-Nelson**
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Massachusetts Institute of Technology

**Goal:** The primary aim of this research is to utilize several naturally occurring, short-lived radionuclides ($^{32}P$, $^{33}P$, $^{7}Be$, $^{234}Th$) within the Gulf of Maine in order to quantify: 1) uptake and regeneration rates of nutrients in dissolved, suspended, and large particulate phases; 2) zooplankton grazing rates; and 3) export of C, N, and P from the euphotic zone.

**Rationale:** The ability to resolve temporally and spatially *in situ* nutrient turnover and export rates will have a tremendous impact on a wide range of investigations. For example, reliable determinations of phosphorus uptake, remineralization and export rate will help resolve the continued debate concerning primary production limitations by N, P, or trace elements and under what conditions these may vary. In addition, our results should increase understanding of the fate of organically bound or biologically active contaminants and heavy metals in the coastal marine environment. Evidence suggests that the predominant transfer of these contaminants from the upper ocean to underlying sediments is biologically mediated. Thus, it is essential to understand the mechanisms that govern or enhance biologically driven particle fluxes to sediments.

**Approach:** If the atmospheric input of cosmogenic $^{32}P$, $^{33}P$, and $^{7}Be$ is known from direct measurement, the ratio of $^{32}P$ and $^{33}P$ to $^{7}Be$ can be directly related to: (1) the known decay rates of these tracers; (2) the particle reactivity of $^{7}Be$; (3) physical mixing and advection; and, most importantly, (4) the uptake ratio of P into particulate pools (*i.e.,* the biological cycle of nutrient uptake and remineralization). Essentially, $^{7}Be$ is used as both a tracer of upper ocean mixing and as a constraint on the input flux of the $^{32}P$ and $^{33}P$ isotopes. Additionally, if $^{234}Th$ is measured, estimates of particle export can be obtained for $^{7}Be$, organic C, and the major nutrients. Seasonal sampling in Wilkinson Basin in the Gulf of Maine will enable accurate temporal resolution of nutrient turnover rates and export.

**Status:** During the last 18 months, substantial progress has been made in the collection and measurement of $^{32}P$ and $^{33}P$ in rainwater and in total dissolved and particulate seawater pools. For the first time, measurement of $^{33}P$ can be accomplished with relatively high efficiency (50-60%) using a liquid scintillation technique newly developed in the course of this research. Measurements of $^{32}P$, $^{33}P$, $^{7}Be$, and $^{210}Pb$ in rainwater have been conducted continuously since March, 1996. In addition to constraining the input ratio of $^{33}P/^{32}P$, measurements suggest that these isotopes can be used as complimentary tracers of air mass residence times and stratospheric composition. One data-gathering cruise was conducted in July, 1996 on Wilkinson Basin in the Gulf of Maine, and four more are scheduled for the Spring and Summer of 1997. Assuming continuous uptake and regeneration, our results indicate that P export was rapid, on the order of one-to-two weeks, and that bacterial recycling of P occurred on the order of one month. Research should be completed by June, 1998.

# CHARACTERIZATION AND EVALUATION OF VERTICAL HYDRAULIC CONDUCTIVITY VARIATIONS AS THEY CONTRIBUTE TO NONIDEAL TRANSPORT BEHAVIOR AT A SUPERFUND SITE

**Julie Elena Blue**
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University of Arizona

**Goal:** To determine possible causes of reduced efficiency of a pump-and-treat operation at a trichloroethene (TCE)-contaminated Superfund site. Influent TCE concentrations in the system have leveled off at approximately 100 ppb, despite significant earlier reductions.

**Rationale:** The study site is an aquifer which serves as the sole source of water for Tucson, Arizona. Thus, the results of this research may suggest alternative remediation strategies for protecting the health and safety of residents of a large metropolitan area. Processes to be investigated here are rate-limited diffusion from low conductivity zones, rate-limited desorption, and rate-limited dissolution from NAPL phase. Since these important processes take place at many sites contaminated with organic pollutants, the research approach and model results may have wide applications.

**Approach:** Pump test results and water level data collected during a dual-well, forced-gradient field experiment were used to obtain a vertically-averaged hydraulic conductivity. Bromide concentration data were used to calibrate a vertically-averaged longitudinal dispersivity in a numerical model. This dispersivity was compared to results from similar field sites. Bromide data collected from a multi-level sampling well were then used to construct a perfectly stratified four-layered model. Travel time calculations were performed and used with Darcy's law and an analytical solution, respectively, to obtain vertically discrete conductivity values for the model. Vertically discrete longitudinal dispersivities were then calibrated.

Two sublayers will be added to the numerical model to obtain a six-layered model which adequately simulates the double peaked behavior observed in two of the layers modeled previously. The velocities from this model will be used in a TCE transport model. Rate-limited diffusion out of the low conductivity zones, delineated by the tracer modeling, will be incorporated into the model through the use of laboratory-determined parameters for rate-limited desorption and intra-particle mass transfer.

The model will be tested by comparison with measured TCE elution data. Recommendations will be formulated regarding alternative remediation strategies, based on which processes are found to contribute most significantly to TCE transport and fate at this site. If the TCE elution data can be accurately matched, it may be appropriate to apply the procedures used here at other, similar sites.

**Status:** Consistent results were obtained from pump tests and homogeneous flow modeling. A four-layered numerical model reduced calibrated longitudinal dispersivity by an order of magnitude. Vertical conductivities have thus been characterized, and the velocities from this model will be used in the TCE model. Prior to this, attempts will be made to improve resolution with the six-layered model. The TCE modeling is expected to be completed by December of 1997.

# COLONIZATION OF LOGGED HABITAT BY CHECKERSPOT BUTTERFLIES

**David A. Boughton**
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University of Texas, Austin

**Goal:** To examine the ecological and behavioral mechanisms structuring the colonization by the butterfly *Euphydryas editha* of highly-disturbed, logged clearings and low-disturbance, selectively-logged forest patches in the Sequoia National Forest in California.

**Rationale:** Small-scale logging does not necessarily destroy habitat of herbivorous insects, but it does alter it. Since insects are an integral component of biodiversity, understanding how an insect population responds to disturbances such as logging helps to predict the impacts of land-use decisions on biodiversity. This project provides a detailed case study for the effects of logging disturbance on one species of herbivorous insect.

**Approach:** In the 1980s, populations of the butterfly inhabited clear-cut patches, where they used the host plant *Collinsia torreyi,* and patches of selectively-logged forest, where they used the host plant *Pedicularis semibarbata* (a hemiparasite of conifers). In 1992, a summer frost destroyed the populations in clearings, and in 1995, insecticide was applied as part of this research to six small patches of wooded habitat. Recolonization of these two types of vacant habitat from extant populations of the butterfly in wooded habitat was studied. Wooded patches of *P. semibarbata* were all recolonized at high density the first year after extinction. Many failed attempts at recolonization of *C. torreyi* in clearings occurred, and only half the patches were colonized in 1996, all at very low density.

A variety of studies were performed to elucidate the mechanisms causing the slow colonization of clearings. Mark-recapture studies revealed that adult butterflies commonly move into clearings during the summer flight season, evidence that spatial barriers did not block recolonization of clearings. However, relative timing of suitability for the two types of patches is different, and this temporal barrier is blocking recolonization of clearings. Experiments showed that adult flight season would occur 1-2 weeks earlier in sunny clearings than in wooded habitat. Because *C. torreyi* senesces and dries out midway through the season, immigrant butterflies from wooded areas reach the patches too late to successfully use this plant, thus failing to colonize. This problem would not occur for populations already established there. Behavioral mechanisms also discouraged colonization of clearings. Adult females experimentally introduced to small patches of each host type found and oviposited on *P. semibarbata* with a higher success rate than *C. torreyi.* This is probably because the butterflies have only incorporated *C. torreyi* into their diet within the last 20 years (since the creation of clear cuts) and have not yet become adapted to it. In summary, both kinds of logging left suitable habitat, but clear cuts were less easily colonized and had populations that were less adapted behaviorally to their host plant and more subject to extinction.

**Status:** Most experiments are completed; however, monitoring of recolonization of clearings continues, and examination of the role of parasitoids in promoting or blocking colonization is planned. Computer simulations of the system will also be designed to investigate the consequences of alternative land-use scenarios on the population dynamics of *E. editha.*

# CARBON CYCLING IN A COLORADO SUBALPINE FOREST

**Dave R. Bowling**
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University of Colorado

**Goal:** To quantify various components of the carbon cycle in a subalpine forest at the ecosystem level, including: photosynthesis; leaf, woody tissue, root, and soil respiration; and emission of hydrocarbons (isoprene, monoterpenes, and methylbutenol, or MBO).

**Rationale:** First, atmospheric carbon dioxide has been steadily increasing since the beginning of the industrial revolution. The amount of $CO_2$ produced by fossil fuel combustion is fairly well quantified and accounts for only 40-60% of total atmospheric increase. The remainder has presumably been absorbed by the terrestrial biosphere or the oceans or both. A thorough understanding of the magnitude and seasonality of terrestrial carbon sinks is imperative to future policy decisions regarding $CO_2$ emissions. Second, hydrocarbon emission by vegetative species has been directly implicated in regional photochemistry, leading in many cases to formation of tropospheric ozone. Coniferous tree species strongly emit monoterpenes and emit isoprene and MBO to a lesser degree. These compounds have the potential to combine with NOx produced in urban areas or by soil microbes, resulting in significant ozone production. Additionally, emission of these compounds may amount to several percent of the carbon fixed in photosynthesis and may therefore be an important part of carbon cycling in this forest.

**Approach:** The field site is a subalpine forest at 3050 m elevation in north-central Colorado, dominated by pine, spruce, and fir. Above-canopy fluxes of $CO_2$, $H_2O$, and biogenic hydrocarbons will be monitored from a 25 m tall instrumentation tower using eddy correlation and relaxed eddy accumulation. Eddy correlation fluxes of $CO_2$ and $H_2O$ will also be measured at the forest floor. $CO_2$ flux through the snowpack in the winter will be monitored using a gradient profile technique. $CO_2$ flux in the summer, as well as foliar and woody tissue respiration, will be measured using enclosures. Accompanying energy balance, forest meteorology, and ecological characterization measurements will also be made. Continuous measurements of these parameters will be made for the duration of the project.

**Status:** Relaxed eddy accumulation (REA) is an emerging technique that has been used to measure isoprene in only one prior study. The use of this method has not been directly validated against eddy correlation. An REA to measure isoprene fluxes was designed, built, and tested against the eddy correlation technique at 40 m height over a deciduous forest in eastern Tennessee in August 1996. Excellent linear agreement between the two techniques ($r^2=0.974$, $n=62$, $p<0.001$) was obtained, establishing the validity of the technique for use with a photochemically reactive gas. Measurements in Colorado will begin in June 1997 and continue indefinitely. The full suite of necessary instrumentation has been purchased, and custom instruments are currently being constructed for the experiment.

# AGRO-INDUSTRIAL DEVELOPMENT, ECONOMIC RESTRUCTURING, AND ENVIRONMENTAL RISK IN THE POST-WAR AMERICAN SOUTH

**William Boyd**
Energy and Resources Group
University of California Berkeley

**Goal:** To better understand the relationships between economic restructuring and environmental risk in the post-World War II rural South. More specifically, the objective is to analyze one of the major drivers of economic and environmental change in the region—referred to here as the "new agro-industries" (primarily, intensive animal agriculture and industrial forestry)—in order to explain how the distribution of environmental risk is implicated in the very process of economic restructuring and agro-industrial development. The major questions posed by this research include: (1) How are agriculture and forestry being industrialized in the post-WWII South? (2) How are these "new agro-industries" organized? (3) What are the major environmental risks, liabilities, and distributional issues associated with these industries? and (4) What are the major regulatory challenges involved?

**Rationale:** In addition to generating significant economic and environmental change across the region, the model of vertically-integrated agro-industry pioneered in the American South, particularly in the poultry and forest products sectors, is now being replicated with other commodities in other areas. The poultry industry in north Georgia is widely considered to be the paradigm of intensive animal agriculture. Recent court decisions have placed the growing environmental risks generated by the industry's increasing waste stream squarely on the regulatory agenda. The pulp and paper industry in south Georgia has been a leader in the plantation-based model of industrial forestry throughout the postwar period, and many of the environmental risks associated with the industry continue to pose major distributional and regulatory issues. Understanding the historical development and organizational structure of these agro-industries and the types of growth trajectories they are following is critical to analysis of the associated environmental risks and corresponding socio-economic and distributional issues. These agro-industries have had major impacts on rural communities and rural environments throughout the South, and they pose significant regulatory challenges, yet they are understudied.

**Approach:** This project is based on a combination of regional analysis and case-study research. Research techniques include time-series data collection and analysis, documentary and archival research, and interviews. The overall intention is to situate case studies of agro-industries in particular communities within a broader regional and historical context, using the case studies to "ground" the larger argument by providing insight into local processes and mechanisms of economic and environmental change. Two case studies are currently planned, each of which will involve a four- to six-month field research component. The first case study will be on the vertically-integrated poultry industry in north Georgia. The second case study will be on the pulp and paper industry in south Georgia. These case studies will focus on the following aspects of each agro-industry: (1) how and why the particular agro-industry emerged in these particular places; (2) the environmental and socioeconomic implications of the industry; and (3) how and to what extent the environmental risks and liabilities associated with the industry have been differentially distributed.

**Status:** A review of the relevant literature has been completed, and a statistical compendium on economic and environmental trends in the postwar South has been compiled, with particular attention to agro-industrial development in the state of Georgia. The first case study on the poultry industry in north Georgia has also been completed. The second case study is slated for completion by the end of the summer. A number of follow-up trips are also planned for next fall/winter (1997-98). Research is expected to be complete by summer 1998.

# ROLE OF REACTIVE OXYGEN SPECIES IN EASTERN OYSTER IMMUNOLOGY

**Lisa Bramble**
Marine-Estuarine-Environmental Sciences Program
University of Maryland

**Goal:** The goal of this research project is to assess the contribution of reactive oxygen species (ROS) produced by phagocytic blood cells (hemocytes) of the eastern oyster, *Crassostrea virginica,* to bactericidal activity.

**Rationale:** East coast populations of *C. virginica* have been in serious decline over the last 30 years due largely to two protozoan pathogens: *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (dermo). The immune response of oysters is not clearly defined on a cellular level. By analogy with mammalian immune reactions, it has been proposed that ROS (*e.g.*, $O_2^-$, $H_2O_2$, $OCl^-$) produced by oyster hemocytes similarly contribute to host internal defense. An understanding of the components of a successful immune response may assist in the development of management strategies that seek to minimize the impact of pathogens on wild and cultured oyster populations.

**Approach:** The initial step was to determine if exposure of oyster hemocytes to *Listonella anguillarum,* an opportunistic bacterial pathogen, stimulates ROS production. Lucigenin- and luminol-enhanced chemiluminescence (CL) were used to quantify hemocyte-derived $O_2^-$ and $H_2O_2$/$OCl^-$, respectively. *L. anguillarum* failed to elicit hemocyte lucigenin and luminol CL. This finding is inconsistent with a proposed role of ROS in bacterial killing by oyster hemocytes. A potential mechanism of bacterial pathogenicity is suppression of phagocyte-generated ROS by the activity of antioxidant enzymes, particularly superoxide dismutase (SOD), in conjunction with catalase. The use of a SOD inhibitor (sodium nitroprusside) and a comparison of the effects of a catalase-positive and -negative species led to the hypothesis that bacterial SOD and catalase suppress hemocyte-derived lucigenin CL and luminol CL, respectively.

A comparison was then made of the ability of oyster and striped bass *(Morone saxatilis)* phagocytes to produce luminol CL in response to the model stimulant zymosan (yeast cell wall preparation) and to *L. anguillarum.* The data suggest that bass phagocytes have a substantially greater capacity to generate ROS than do oyster hemocytes, which enables the bass phagocytes to exceed bacterial antioxidant capability. This proposition will be addressed in a subsequent study in which bactericidal assays will be conducted in the presence and absence of the NADPH-oxidase inhibitor diphenyleneiodonium. (NADPH-oxidase is the phagocyte-associated enzyme that initiates the process of ROS formation.) Results are expected to suggest that while ROS contribute to bactericidal activity of striped bass phagocytes, ROS do not play a role in the *in vitro* killing of *L. anguillarum* by oyster hemocytes. This finding would be among the strongest evidence to date that ROS may not be involved in microbicidal activity mediated by eastern oyster hemocytes.

**Status:** The initial study has been submitted for publication. The comparative study of oyster and bass phagocyte-generated luminol CL is currently being conducted. Because results generally oppose the hypothesis that oyster hemocyte-derived ROS are microbicidal, a positive model (i.e., striped bass phagocytes) is being used to help substantiate data of a negative nature. Tentatively, this research is expected to be completed by January 1999.

# PARTICLES, CLOUDS, AND CLIMATE

**Fred Brechtel**
Department of Atmospheric Science
Colorado State University

**Goal:** To determine the relationships between particulate matter chemical and physical properties and their cloud droplet nucleating capability. Research will attempt to answer the following questions: (1) Can the cloud condensation nucleus (CCN) activity spectrum be derived from measurements of the hygroscopic growth of aerosol particles? (2) How do changes in laboratory particle bulk and surface chemical composition affect the cloud droplet nucleating ability of the particle? (3) Can laboratory aerosol/CCN study results be related to observations of aerosol and cloud characteristics from a remote marine region during clean and continentally perturbed conditions?

**Rationale:** Clouds influence climate by interacting with incoming solar radiation and by absorbing outgoing terrestrial radiation. The microphysical properties of clouds that determine their radiative characteristics are influenced by the properties of the CCN upon which cloud droplets form. Clouds in remote regions would be most susceptible to changes in particle properties associated with continental influence. Changes in particle bulk and surface chemical composition, associated with natural and anthropogenic influences, thus could alter cloud radiative properties and climate by altering the CCN spectrum.

**Approach:** The first phase of this work has been completed and involved observations of aerosol characteristics over the remote Southern Ocean between November 21 and December 13, 1995, during the first IGAC Aerosol Characterization Experiment (ACE-1). The measurements are being analyzed to separate the data set into clean marine and continentally influenced time periods. Particle properties in both types of air masses will then be compared.

The second phase of this work is aimed at developing a theoretical treatment of CCN activity based on simple measurements of particle characteristics. The theory can be used to link changes in particle properties to effects on clouds. The approach involves laboratory studies using a Tandem Differential Mobility Analyzer (TDMA) with humidity control to expose monodisperse (composed of particles of uniform size) aerosol populations to known humidities and measure their hygroscopic (*i.e.*, humidity-related) growth. Simultaneously, the critical supersaturation and CCN concentration are measured with a thermal diffusion chamber. Results from the hygroscopic measurements are used to derive "supersaturation equivalent" coefficients that allow the CCN concentration and critical supersaturation to be derived from the TDMA results. The derived CCN parameters are then compared to observations made with the thermal diffusion chamber. This technique thus relates specific particle properties, size, and effective chemical composition to particle hygroscopic growth and cloud droplet nucleating capability. Laboratory findings will be used to predict the CCN activity of aerosol observed in ACE-1.

**Status:** ACE-1 observations and data processing have been completed and a manuscript is in preparation. Theoretical and laboratory studies of the aerosol/CCN relationships are ongoing. The research will be completed during 1997.

# QUINONE-INDUCED MITOCHONDRIAL DYSFUNCTION

**Laura A. Briggs**
Environmental Science and Health Program
University of Nevada

**Goal:** To determine if exposure to quinones commonly found in urban air and cigarette smoke results in compromised mitochondrial function, decreased respiratory capacity, and damage to mitochondrial DNA (mtDNA).

**Rationale:** Humans are continuously exposed to a number of xenobiotic quinone compounds which can potentially lead to a number of disease states. These studies will attempt to further elucidate the mechanisms of quinone toxicity and identify mitochondria, the source of cellular energy , as a potential target. These data will be valuable to attempts at understanding the biological impact of exposure to these pollutants.

**Approach:** The quinones which will be used in these studies include p-benzoquinone, 2-methyl-p-benzoquinone, 1,4 napthoquinone, and 2,3,6-trimethyl-1,4, naphthoquinone. The models for these studies are Chinese Hamster Ovary (CHO) cells and Balb/c mice. Interactions with mitochondrial DNA will be determined by assessing the degree of conformational change from the supercoiled to the relaxed state under both *in vitro* and *in vivo* conditions. Mitochondrial DNA isolated from exposed models will be analyzed for effects of exposure on ATP levels. (ATP, or adenosine triphosphate, is the compound within mitochondria that supplies energy for cellular processes.) Heart and liver tissues as well as CHO cells will be assessed for ATP levels. Oxidative damage to the mtDNA will be ascertained and any existing point mutations will be noted. Treated samples will be compared with untreated samples to differentiate between polymorphisms and pathogenic mutations.

**Status:** Dose-response curves are currently being developed to determine appropriate dosages. In addition, protocols for optimizing the SSCP analysis for particular fragments are being developed. The conformational change analysis portion of the study is expected to begin in the near future. I expect to complete this research in the summer of 1999.

# THE INFLUENCE OF REDOX POTENTIAL ON SPECIATION OF HEAVY METALS AND NUTRIENTS IN COMPOST-AMENDED SOILS

**Geoffrey A. Brown**
Department of Soil, Crop and Atmospheric Sciences
Cornell University

**Goal:** Two questions were addressed in this research: (1) What oxidation-reduction (redox) conditions are experienced in agricultural soils to which wastes may be applied? (2) Under the redox conditions noted, what changes in trace and toxic metal speciation occur in waste-amended soils which may effect the environmental fate of metals?

**Rationale:** Increasing amounts of composted municipal solid waste and sewage sludge with elevated concentrations of heavy metals are being land-applied in the United States. In the Northeast, many soils experience alternating periods of wetting and drying which may influence soil redox potential. Redox potential is one of the key parameters controlling metal speciation. Thus, fluctuations in soil redox potential may significantly effect the bioavailability of metals added in land-applied wastes. Redox-influenced changes in metals speciation are also relevant in hazardous waste assessment and remediation, wastewater treatment and wetlands biogeochemistry.

**Approach:** For two years, redox potential, depth to groundwater, and soil temperature were monitored in nine soils in or adjacent to agricultural fields suitable for application of wastes. Soils varied in chemical and physical characteristics due to differences in parent material and drainage class. Four soils which experienced a wide range of redox potential conditions were selected for the laboratory component of the study. In the lab, soils were amended with agricultural rates of composted MSW and sewage sludge and then mixed in soil:water slurries at a 1:5 ratio (by weight). Soil-water slurries were utilized to allow homogenization and control of redox potential. Continuous bubbling of $N_2$ gas and microbial oxidation of soil and compost organic matter created a strong tendency for the slurry system to become reduced. Computer-controlled introduction of air countered this tendency and allowed redox to be controlled within 10 mV of the set value. Redox potential was controlled at 4 levels: -200 mV, 0 mV, +225 mV and 450 mV, representing the strongly-reduced to moderately-oxidized conditions noted in the field. The vessels were maintained at each redox level for 7 days. At the end of this time, slurry samples were collected, and redox was then raised to the next level. Slurry samples were sequentially extracted and analyzed to determine nutrient and metal concentrations in the following fractions: water soluble; exchangeable; sorbed or co-precipitated on hydrous Fe and Mn oxides; organic-bound; and residual.

**Status:** The field and laboratory components of this investigation have been completed.

# HALOPHYTES FOR THE TREATMENT OF SALINE AQUACULTURE EFFLUENT

**J. Jed Brown**
Department of Wildlife and Fisheries Sciences
University of Arizona

**Goal:** To determine whether halophytes, which are salt tolerant salt marsh plants, can be used to clean up the wastewater generated by marine aquaculture farms. The halophytes used in this research have been domesticated for use as crop plants and could provide an economic return to the grower while simultaneously serving as a biofilter for the effluent.

**Rationale:** Fish and shrimp farming has grown dramatically in recent years. Initially aquaculture was viewed by most people as a clean technology that would help provide protein for the world's population and simultaneously reduce fishing pressure on over fished wild stocks. However, many environmental problems have come to light recently related to fish and shrimp production. Chief among these is the problem of effluent (wastewater) disposal. Water exchange is required to maintain water quality in shrimp and fish ponds. The wastewater from the ponds is typically high in dissolved inorganic nutrients and organic matter. If this water is discharged untreated into the adjacent water body, the water quality of the receiving water body will deteriorate and eutrophication (the process by which a body of water becomes rich in dissolves nutrients such as phosphates but deficient in dissolved oxygen) will occur. Low-cost but effective technologies are needed to treat the saline wastewater generated by shrimp and marine fish farms.

**Approach:** Greenhouse studies have been conducted using lysimeters (draining, soil-filled containers) in order to perform mass-balance studies on the nutrient removal capacity of halophytes. So far, one study has been completed using lysimeters in which filtering capacity of three species of halophytes was investigated using wastewater of three salinities. Plants were irrigated weekly with wastewater to replace the amount of water lost to evapotranspiration during the previous week and to provide a leaching fraction of 0.3. The leaching fraction is the portion of the total applied water that leaches below the plant root zone and carries with it excess salts. This fraction is required to prevent the build up of salts in the root zone to levels that would inhibit plant growth. Across all treatments, the plant-soil system removed >95% of the applied nitrogen and phosphorus from the wastewater stream despite the leaching fraction. However, the total volume of water that could be processed by the plant decreased as the salinity increased. This led to a second experiment to determine whether satisfactory effluent scrubbing could be obtained at salinities similar to full strength seawater. One halophyte species and one irrigation water salinity (30 ppt) were used. Treatments were five different irrigation rates applied three times per week at rates varying from 50% to 250% of pan evaporation. Good plant growth was obtained at all five irrigation rates, but plant growth and filtering performance increased significantly with increased rates.

**Status:** The majority of the research has been completed. A manuscript describing the first experiment is to be submitted for publication by March 30. The second experiment is scheduled to be written up and submitted for publication within the next three months. It is hoped that research will be complete by December 1997.

# DETECTING MULTIPLE POLLUTANTS WITH A SINGLE PROBE

**Audra M. Bullock**
Department of Electrical and Computer Engineering
Old Dominion University

**Goal:** To create a system capable of making real-time, non-intrusive, simultaneous concentration measurements of more than one pollutant species in gaseous and liquid media with resolution to parts per trillion, using a single laser probe.

**Rationale:** Multi-species detection with a single probe is an extremely valuable technology. The ability to detect the concentration of several pollutants with a single measurement saves time and eliminates the expense of owning and operating duplicate equipment designed for different pollutant species.

**Approach:** A non-intrusive measurement technique known as absorption spectroscopy is used, which alone is not very sensitive but, when coupled with a few signal processing tricks, results in extreme sensitivity without the need for complex or expensive equipment. This technique, known as Wavelength Modulation absorption spectroscopy, has the potential for detection of multiple pollutant species and their concentrations from parts per billion (ppb) to parts per trillion (ppt).

The basic technique of absorption spectroscopy is quite simple. A laser probe is sent through the sample (liquid or gas), with a characteristic energy (wavelength) that can be absorbed by the species being probed. The laser beam intensity is then detected after passing through the sample where it has less intensity than the light entering the sample. The change in intensity is proportional to the density or concentration of the absorbing species and the energy (wavelength) of the light identifies the species.

In general, individual molecules have a unique absorption spectrum by which they can be identified, somewhat like a fingerprint. Often the spectrum of many different molecules overlap, creating an interference in the detection of a single species. A means of deconvolving the spectra within the region of focus (the wavelength region being probed) has been developed, to isolate one species of interest or all those which are overlapping. This capability, together with the real-time nature of this method, means that the dynamic concentrations of CO and $CO_2$ in air, for example, or pollutants such as heavy metals in ground water can be detected with sensitivity that surpasses most currently used apparatuses.

**Status:** A series of measurements has been completed which show that the measurement methods most commonly used by researchers are not the most sensitive for multi-species detection. To date, experiments have been performed only with oxygen, a molecule that is a very weak absorber, difficult to probe, and has a congested spectrum in some regions. In the near future, research will focus on molecules in the presence of water vapor and on air and water pollutant species of interest to EPA.

# UTILIZATION OF CARBON DIOXIDE AS A REACTION MEDIUM FOR THE SYNTHESIS OF FLUOROPOLYMERS

**W. Clayton Bunyard**
Department of Chemistry
University of North Carolina at Chapel Hill

**Goal:** To determine the viability of supercritical and liquid carbon dioxide as an environmentally responsible reaction medium for the synthesis of fluoropolymers.

**Rationale:** Fluoropolymers comprise a class of commercially important specialty polymers due to their exceptional high thermal and chemical stabilities. Due to the inherent insolubility of fluorinated monomers and polymers in traditional organic solvents, fluoropolymers have been typically synthesized in industry using chlorofluorocarbon (CFC) solvents. The recent ban imposed by the Montreal Protocol on further utilization of CFCs has required the development of new solvent systems for fluoropolymer synthesis. This daunting task has resulted in the development of numerous CFC alternatives such as perfluorocarbons, perfluorinated heterocycles, hydrofluoroethers, hydrochlorofluorocarbons (HCFCs), and hydrofluorocarbons (HFCs). Unfortunately, many of these solvents are industrially impractical due to either the lack of current bulk availability or high cost. HCFCs are presently serving as a short-term CFC replacements but their use will be phased out around 2030. Thus, there is a need for the development of long term CFC alternatives.

**Approach:** The utilization of liquid and supercritical carbon dioxide ($CO_2$) as reaction mediums for fluoropolymer synthesis has been investigated. Carbon dioxide has several advantages as a reaction medium: it is inexpensive, environmentally benign, non-toxic, and inert to free radical reactions. We have successfully synthesized a number of fluoropolymers in $CO_2$, most notably, the commercially important homopolymer and copolymers of tetrafluoroethylene. The current emphasis of this project involves perfluoropolyether synthesis in $CO_2$. Industrially, perfluoropolyethers derived from the photooxidation of tetrafluoroethylene are synthesized in dichlorodifluoromethane as a reaction medium. Due to the high solubility of perfluoropolyethers, fluoroolefins, and oxygen in $CO_2$, it should be a promising alternative reaction medium.

**Status:** Initial results focused on the photooxidation of hexafluoropropylene, which illustrated that this chemistry was indeed compatible with $CO_2$. The current goals for this project involve improving upon initial results and optimizing the reaction conditions for the synthesis of high molecular weight perfluoropolyethers in high yields. The influence of the inherently low viscosity of liquid $CO_2$ on the polymerization reaction and the resultant polymer structure is also being investigated.

# AN EVALUATION OF POPULATION DIFFERENTIATION FOR TWO ANURANS (*RANA LUTEIVENTRIS* AND *HYLA REGILLA*): THE LIMITS OF GENETIC INFERENCE

**Douglas Calt**
Department of Zoology
Washington State University

**Goal:** To assess the possibility of using molecular markers as an indirect method for studying local and regional population dynamics relative to habitat fragmentation.

**Rationale:** There are many attempts underway to explain worldwide declines in amphibian populations. All efforts acknowledge that habitat destruction and fragmentation are probably a primary contributor, but there are few tools available to assess effects of habitat disturbance on local and regional population dynamics. Conventional approaches (mark/recapture and telemetry) are very expensive and limited by field logistics. Genetic markers may provide a relatively inexpensive and non-intrusive method for studying relationships between migration rates among local populations and landscape structure.

**Approach:** Three questions were addressed: (1) At what scale can population differentiation be detected? (2) How might the interpretation of that differentiation be affected by the molecular marker that is used? and (3) Are relative levels of differentiation meaningful? The last question is important if this approach is to be used to understanding the fates of individual populations. Tissue samples (toe clips) were collected from local populations in the Selkirk Mountains (WA) and from five other sites in WA and Idaho. Three microsatellite markers for *R. luteiventris* and four markers for *H. regilla* were developed by cloning frog DNA, screening clones for microsatellite insert, and generating primers for polymerase chain reaction (PCR). The latter method allowed generation of genotypic data for each marker using small quantities of template DNA. Characteristics of the microsatellite markers were evaluated, and they appeared to follow a general stepwise mutation model, albeit with very high variances. These high variances along with presence of null alleles make the microsatellite markers less useful for broad scale estimates of phylogeographic patterns. Smaller scale estimates of differentiation were mixed with half of the markers indicating no population subdivision.

A multilocus detection method (Southern blot) was also used for small scales and significant differentiation was found for three different probes. Multilocus and single locus methods reveal different types of genetic variation. More microsatellite markers are needed to determine whether the multilocus patterns represent genuine genetic differentiation or simply an artifact of these markers. A stochastic population model was designed to evaluate the ability to make inferences at small scales. It is generally possible to detect mean differentiation after a short period of isolation, but this is a function of population size and the number of markers employed. The relative degree of differentiation can be highly variable, limiting the usefulness of indirect measurements with genetic markers for understanding the fates of individual populations.

**Status:** Lab work is completed and data are currently being analyzed. Dissertation defense is scheduled for 22 April 1997.

# COMPLEX DYNAMICS OF BOREAL PERMAFROST PEATLANDS ACROSS A CLIMATE GRADIENT

**Philip Camill**
Department of Botany
Duke University

**Goal:** Ecosystems such as boreal permafrost peatlands will probably exhibit complex, nonlinear dynamics during a climatic warming because of local processes that create microclimatic conditions quite different from the regional climate change. This research, conducted in boreal permafrost peatlands across a 4°C climate gradient, addresses the following questions: (1) how do vegetation and carbon dynamics differ across a regional climate gradient? and (2) to what extent do local processes stop or stabilize melting?

**Rationale:** Boreal systems of northern high latitudes contain upwards of 33% of the world's terrestrial carbon, half of which is stored as peat. Scientists are concerned about how this large store of carbon will react to a potential onset of a 4°C warming at these latitudes in the next century due to planetary climate warming. Many speculate that peat reserves will begin to decompose in warmer temperatures, thereby emitting more $CO_2$ to the atmosphere and accelerating greenhouse warming. This assumption depends on the rate at which systems change during warming. Research so far suggests that boreal peatland carbon dynamics are intimately related to vegetation dynamics and complex vegetation responses indicate that carbon responses will likely not be so predictable.

**Approach:** Melting rates of permafrost peatland features known as collapse scars, estimated using tree rings, suggest that permafrost is melting faster in warmer sites. However, local microclimatic conditions of permafrost peatland collapse scars are influenced by azimuth and the presence of the hummock forming peat moss, *Sphagnumfuscum,* both of which can cause melting to stop. Southern edges of collapse scars are frequently more stable than northern edges, because southern edges receive less solar insulation. Low thermal diffusivity of *S. fuscum* on permafrost plateaus appears to insulate the subsurface and stabilize melting collapse scars. Southern azimuth and hummock-forming *Sphagnum* are sufficient to stabilize a melting scar and will likely introduce nonlinear successional dynamics during climate warming.

**Status:** Modeling of landscapes to predict the complex effects that local processes will have on the dynamics of boreal permafrost peatlands during climate warming has begun, using the empirically determined rates and processes described above.

# KERATINASE APPLICATION: BRIDGING THE GAP BETWEEN THEORY AND IMPLEMENTATION

**Scott D. Carter**
Department of Nutrition
North Carolina State University

**Goal:** To develop the keratinase enzyme for large scale field application with the intent of reducing the large amount of keratin waste produced by the poultry industry each year.

**Rationale:** In 1992, the U.S. poultry industry produced 54 million tons of manure and 15 million tons of scrape manure and litter, a substantial portion of which was feather keratin waste. Keratinase, an extracellular protease (enzyme that breaks down proteins) produced by *Bacillus lichen formis* PWD-1 has the ability to degrade this keratin waste. Following application of the enzyme, this waste can be recycled by feeding it to chickens as a high protein supplement. If practical methods of enzyme production and application can be developed and implemented, the poultry industry could utilize this technology to greatly reduce the overall production of feather keratin waste per year.

**Approach:** 1) Quantitation of application parameters: A double protease assay using trypsin and keratinase is used to determine the *in vitro* hydrolysis of feather keratin under a variety of application parameters. Using this assay, the influence of sample pH, fat content, temperature, and other controllable parameters can be determined. 2) Optimization of fermentation conditions: Wild type PWD-1 has largely been optimized for fermentation, and methods used for determining optimal parameters are also being used for optimization of mutants that overproduce keratinase. Factors influencing optimal enzyme production include age of inoculum at each scale-up step; pH level during fermentation; level of dissolved oxygen; level of feather keratin substrate; and temperature. 3) Enzyme application: Parameters determined to be important using a small-scale assay are used to predict scale-up parameters for producing the enzyme in a 100 liter, in-house fermentation used for large scale application. Large scale application consists of applying enzyme under predicted parameters to commercial feather meal as a protein supplement in chicken feed. Chickens are fed this diet from 1-3 weeks of age, with body weight gain and feed/gain ratios taken as quantitative data. Application parameters are evaluated as weight gain and feed/gain ratios *vs.* a soy bean meal positive control diet.

**Status:** To date, the *in vitro* assay is routinely used to test different parameters on enzyme application. Twelve keratinase over-producing mutants have been isolated, and the four best mutants are being optimized for fermentation. Optimization is complete for the best mutant. Keratinase is being routinely produced by large-scale fermentation, and application of this enzyme is underway, with two chicken feeding trials already completed. Another two to three feeding trials are planned, followed by a large-scale, 7-week feeding trial once optimal application parameters are determined. Completion of this project is expected by February 1998.

# EXAMINING THE INTERACTIONS BETWEEN ECONOMIC POLICIES AND DEFORESTATION IN THE BRAZILIAN AMAZON: FEEDBACK EFFECTS OF LAND DEGRADATION ON LAND-USE PATTERNS

**Andrea Cattaneo**
Department of Geography and Environmental Engineering
Johns Hopkins University

**Goal:** To assess the impacts of economic policies on deforestation and land degradation in Brazil and the feedback effects of biophysical changes on economic activities. Questions to be addressed are: (1) How do trade liberalization, and agricultural tax and support policies affect deforestation and land degradation? (2) What impact do policies affecting labor migration have on the environment? By considering the environmental feedback onto the economy, it is hoped that the ways in which bio-physical processes and economic incentives interact to determine land use changes and economic/environmental outcomes will be clarified.

**Rationale:** By including ecological processes in a dynamic economic framework, this approach may help policy makers resolve the ambiguities they face concerning the operational meaning of sustainability. This research could also contribute to current efforts aimed at improving natural resources accounting and valuation in the context of welfare economics analyses.

**Approach:** The modeling exercise will start from the creation of a computable general equilibrium (CGE) model of Brazil, with agricultural production disaggregated by region (Amazon, Northeast, Rest of Brazil). Land will be a regional factor of production differentiated on the basis of cover, which means it will be available in a number of "states": pristine forest, secondary forest, arable land, grassland. Land may change state through two different processes: (1) land conversion intentionally brought about by economic agents (represented in the model by a deforestation sector), and (2) land transformation due to environmental processes linked to a given economic use.

The second step integrates the CGE modeling component with a bio-physical model. The land transformation process will be represented as a first-order stationary Markov process with land use as an exogenous variable. The transition probabilities will be estimated from a sample of transitions (conditional on land use) occurring between two points in time. Depending on data availability, this could be done by: (1) using land use maps and applying a maximum likelihood method for explanatory variables other than land state to plots with comparable levels; (2) running Monte Carlo simulations using crop models adapted for tropical areas (DSSAT, EPIC, or CENTURY); and (3) using data on production practices from farm level surveys.

A recursive application of a static CGE model will be adopted, so that successive runs come to represent successive points in time. Although time-dependent variables, such as aggregate capital stocks, labor force, and total factor productivity are updated between periods, they remain exogenously set within each period. The exogenous variables will be obtained from the Brazilian National Development Bank. The land stocks will be updated by extracting land type stocks (affected through land conversion) from the CGE solution, applying the land transformation component to obtain new land type stocks, and feeding these into the CGE model for the following year.

**Status:** A small demonstration model is complete. It includes three agricultural sectors for two regions: the Amazon and the Rest of Brazil. More data are required to represent the entire Brazilian situation.

# NATURAL ORGANIC MATTER AND PARTICLE FOULING OF SPIRAL-WOUND NANOFILTRATION MEMBRANE ELEMENTS

**Tory L. Champlin**
Department of Civil Engineering
Colorado State University

**Goal:** To develop a fundamental understanding of membrane fouling caused by natural organic matter (NOM) and particles in water.

**Rationale:** With the anticipated promulgation of the Disinfectants and Disinfection By-Product (D/DBP) Rule, water treatment utilities will face lower maximum contaminant levels (MCLs) for both trihalomethanes (THMs) and haloacetic acids (HAAs). Membrane filtration is currently one technology being considered to meet these new standards. Membranes that accomplish nanofiltration have been shown to effectively remove DBP precursors.

A major factor limiting the application of membrane filtration is the problem of fouling. Fouling is observed as a decline in water production as a result of matter deposited on the surface and pores of the membrane. Research relating to fouling is limited, and questions remain regarding the mechanisms and significance of fouling caused by naturally occurring substances in water. Understanding membrane fouling is essential in selecting effective strategies for its control. The research is intended to provide this knowledge as related to NOM and particles.

**Approach:** Empirical investigations have examined deposition and fouling at a macroscopic scale through recirculation experiments using a membrane pilot-plant. Experiments have used synthetic waters where fouling characteristics have been controlled. These waters were created by spiking permeate water with concentrated NOM and particles collected from the same surface water source. Four NOM concentrations (0, 5, 15, and 25 mg/L as total organic carbon (TOC)) and three particle sizes ( no added ancillary particles, 0.5-1.0 $\mu$ m, and >1.0 $\mu$ m) are being tested, for a total of 12 experiments. Synthetic waters were recirculated through the spiral-wound membrane elements for 28 days to allow both deposition and fouling by NOM and particles to be observed. Deposition was detected by the loss of NOM and particles in the recirculated water. Fouling was observed through declining water flux rates and decreasing mass transfer coefficients.

Analytical investigations have examined deposition at a microscopic scale through visual inspection of dissected membrane elements and electron microscopic examinations of membrane specimens. Immediately following pilot experiments, membrane elements have been dissected and inspected for deposited matter. Membrane specimens from various locations within the elements were examined using the scanning electron microscope (SEM). Photo micrographs were taken to reveal the presence or absence of deposited matter. Energy dispersive X-ray microanalysis, performed in conjunction with the SEM, was used to identify the deposited matter.

**Status:** As of March, 1997, seven experiments have been completed. Results indicate that particle deposition is significant for spiral-wound membrane elements, and the rate of particle deposition decreases with particle size. NOM, which acts like an adhesive, increases the rate of particle deposition for all size ranges. NOM deposition, however, is not as significant as the literature would suggest. Although deposition of particles and NOM were apparent from empirical and analytical investigations, there was insufficient mass of both in the recirculated waters to cause a decline in water flux rates and mass transfer coefficients. The remaining experiments are expected to be completed in the Fall of 1997.

# DISPUTE RESOLUTION AND CITIZEN PARTICIPATION IN WILDLIFE MANAGEMENT DECISION MAKING

**Lisa Chase**
Department of Natural Resources
Cornell University

**Goal**: To develop, apply, and evaluate a conceptual framework for assessing both procedures and outcomes of decision making in wildlife management. This project will explore methods for deciding which types of citizen involvement are optimal in different situations, given contextual constraints including resources and authority. In particular, this study will focus on approaches to stakeholder involvement that emphasize educational communication, consensus-building, and the potential for joint gains.

**Rationale**: Citizen demand for greater involvement and agency accountability in wildlife management have increased dramatically in recent decades. At the same time, the diversity of people interested in wildlife issues has expanded from the traditional base of hunters and farmers to include stakeholder groups as varied as motorists, animal rights activists, and the tourism industry. Thus, a major concern of wildlife managers today is weighting input from various stakeholder groups and resolving conflicts of interest among them. Research that systematically examines and evaluates approaches to dispute resolution and citizen participation is needed to improve agency decision-making. While the cases examined in this study pertain to wildlife management in the United States, the implications can be generalized to improve decision-making in a variety of natural resource management issues throughout the world.

**Approach**: A conceptual framework for assessing procedures and outcomes of stakeholder involvement approaches will be developed integrating theories from several disciplines including economics, political science, law, education, and philosophy. The framework will consist of a typology of approaches for incorporating citizen input as well as evaluative criteria such as fairness, validity, efficiency, stability, and transferability. Methods for measuring the criteria in relation to specific situations will be developed. The framework will then be used to examine a variety of case studies. Results of this research will provide insights into decisions regarding various public involvement approaches, mechanisms by which weighting of stakeholder input occurs, and the potential for educational communication to improve decision making. Insights derived through contextual analyses will be used to evaluate the conceptual framework and inform theoretical models of decision-making, as well as to contribute methodologically to the techniques for understanding and managing controversial situations.

**Status**: A conceptual framework is being developed concurrent with explorations of wildlife management conflict case studies that can be assessed using the framework. In the next year, course work will be completed and analysis of selected cases will begin. Research is expected to be complete within three years.

# RISK COMMUNICATION AS AN ORGANIZATIONAL RESPONSE

**Caron Chess**
Department of Environmental Studies
State University of New York College of Environmental Science and Forestry

**Goal:** To examine the extent to which organizational theory, specifically resource dependence (RD) theory, explains the use of risk communication by chemical industry plant managers. RD theory suggests that organizations both create and respond to their external environments. For the purposes of this research, the term "environment" refers to social context rather than the biophysical environment. Questions to be addressed include: (1) To what extent can the use of risk communication be explained as an organizational interaction with the external environment? (2) What risk communication methods do organizations use to respond to the external environment? and (3) How does the form of organizational coupling (the extent to which organizational units are bound to each other) affect the interaction between organization and environment?

**Rationale:** Increasingly, chemical manufacturing plants are turning to risk communication as a means of dealing with local concerns about environmental issues. However, risk communication practice and research have focussed on individual or community-level units of analysis and neglected organizational ones. The answers to the organizational questions posed above will help to explain variations in chemical manufacturing site responses to pressures from external stakeholders. Exploration of the structural links between public relations and environmental management units may explain why some plant sites are more responsive than others to the concerns of external stakeholders.

**Approach:** Between 1990 and 1991, qualitative data were collected on three case studies of industry risk communication programs that were ahead of the industry curve. The cases were selected based on six criteria, the most important being conformance to the recommendations of *Improving Risk Communication*, a 1989 National Research Council report. The three cases represent three levels of organizational complexity: 1) Sybron Chemicals—a small plant that developed a risk communication program after a chemical release that led to the evacuation of 60 people; 2) Rohm and Haas's Bristol plant—a mid-sized multinational plant that initiated a risk communication program after a newspaper disclosed the presence of a hazardous waste landfill on the site; and 3) Texas Ship Channel Collaboration—a cooperative risk communication effort by 80 companies that resulted from growing community concern in the wake of major accidents. Interviews for each case were conducted with approximately 12 personnel involved with the risk communication program (from hourly operators to CEOs), using an interview protocol that dealt with a range of organizational issues. This research analyzes case study descriptions, derived from collected data, from a theoretical perspective. Patterns in the descriptions that tie to RD theory are being examined. The next step in the analysis is iterative and interactive, moving from lower level patterns to theory, and from theory to patterns.

**Status:** Theory and description are currently being matched. This process will continue throughout the research write-up, which will begin by April. The dissertation will be completed in Fall 1997.

# ANALYSIS OF REGIONAL CONTROL STRATEGIES TO MEET ALTERNATIVE OZONE STANDARDS IN THE NORTHEAST

**Karen Chess**
Department of Mechanical Engineering
Department of Engineering and Public Policy
Carnegie Mellon University

**Goal:** To investigate the effect of the potential change in the ozone National Ambient Air Quality Standard (from 1 hr, 0.12 ppm to 8 hr, 0.08 ppm) on NOx and VOC control requirements in the Northeast based on regional control strategies.

**Rationale:** Current strategies to comply with the ozone NAAQS are based on the 1-hour average peak ozone, while the potential new standard (proposed by the EPA in November 1996) is based on the 8-hour average. This research uses air quality modeling of the Northeast to examine the effectiveness of strategies based on the current standard in achieving compliance with the new standard, as well as to systematically explore the criteria used for control strategy design. The results are relevant to Northeastern state and regional air quality managers as they plan for compliance with the new standard and will advance the methods of control strategy design.

**Approach:** In this research, the Urban-Regional Multiscale (URM) model is used to simulate the formation, transport, and fate of ozone and its precursor emissions in the Northeast under uniform NOx and VOC control conditions. The URM model is a comprehensive, grid-based airshed model developed at Carnegie Mellon University which utilizes fine horizontal grid resolution (4.6 km) over critical urban centers and coarser grid resolution over other areas to maintain computational efficiency. The URM model is being applied using a meteorological description of the July 2-14, 1988 ozone episode over the ROMNET 2.1 Northeast domain and using the OTC 2005 Revised Base Case emissions inventory (obtained from the EPA).

Twenty-five simulations are planned, corresponding to regionally uniform NOx and VOC control from 0% to 100% in steps of 25% reductions. From these results, response surfaces for 1 hour and 8 hour average daily peak ozone will be developed for three areas within the domain: New York City, Baltimore-Washington, DC, and Pittsburgh. Potential population exposure to ozone levels greater than 0.08 ppm, taken as a reasonable threshold for adverse health effects, is also being computed using gridded population data. Results to date show that response surfaces vary by simulation day, city, and ozone metric, and thus the effectiveness of NOx *vs.* VOC controls in different cities and toward different air quality goals also varies. However, on a day-to-day comparison, the proposed 8 hour, 0.08 ppm standard requires significantly more emission control than the current 1 hour, 0.12 ppm NAAQS. The response surfaces will next be used to explore possible criteria used to choose between NOx and VOC control in reducing ground-level ozone, including relative effectiveness, relative cost effectiveness, and strategy robustness (*i.e.*, the ability of a strategy to reduce ozone on many simulation days instead of just the episode peak).

**Status:** The 25-run matrix of URM simulations will be complete by March 15, 1997. It is anticipated that the analysis of the results, including the optimization of control strategies based on alternative decision criteria, will be completed before the June 1997 STAR Conference.

# LASER-ENHANCED IONIZATION SPECTROSCOPY FOR ULTRATRACE MERCURY DETECTION

**Wendy L. Clevenger**
Department of Chemistry
University of Florida

**Goal**: To detect ultratrace (sub-parts per billion) amounts of mercury in samples of environmental importance (air, water, soil, or tissue) using laser enhanced ionization spectroscopy.

**Rationale**: Laser-enhanced ionization spectroscopy (LEIS) has been proven one of the most sensitive and selective methods for trace metal detection available today. This sensitivity can be further enhanced when an inert atmosphere can be used as the sample introduction method (*e.g.*, a stream of inert gas such as argon) instead of a flame or furnace method. For these reasons, it is an ideal method for detection of mercury in environmental samples, in combination with the well-established method of cold vapor generation of mercury atoms from solution.

The trace detection of mercury in environmental samples is important for many reasons. Mercury is a toxic heavy metal found in the environment not only as a result of unrestricted dumping or industrial releases but also because of atmospheric deposition in pristine areas. Mercury that settles in natural water sediments is converted to its more toxic form, methyl mercury, by bacteria residing in these anaerobic conditions. Methyl mercury increases in concentration as it is carried through the food chain, threatening the health of animals, including endangered species, and humans. It is clear that detection of even ultratrace levels of mercury in air, water, soil, and tissue is of extreme health and environmental importance.

**Approach**: LEIS involves the stepwise excitation of the mercury atom to a high-lying (Rydberg) state near the ionization continuum. From here, collisional ionization of the atom occurs and the resultant electrons are detected as an electrical signal. Three dye lasers, pumped by an excimer laser, are used to excite the atoms. The detection occurs in a quartz cell, into which the mercury atoms are swept by a stream of argon gas. A high voltage (0.5-3.5 kV) is applied between two electrodes to allow collection of the electrons. The signal is amplified and recorded on a digitizing oscilloscope and laptop computer.

**Status**: Fundamental studies of mercury Rydberg states have been carried out in order to find the optimal conditions for ultratrace detection. These studies include the effect of the choice of Rydberg level and temperature on the efficiency of collisional ionization, and the effect of buffer gas pressure and applied high voltage to the spectral lines. In addition, the use of avalanche amplification of the electrical signal was investigated and determined to give a signal three orders of magnitude higher than the signal observed with our normal LEIS setup. With this increase in signal, we have calculated a limit of detection (LOD) of about six mercury atoms.

Future work includes experimentally proving this calculated LOD. We expect to achieve single atom detection using LEIS with avalanche amplification of the signal. The method will then be applied to samples of environmental interest.

# AUGMENTATIVE BIOLOGICAL CONTROL WITH PREDATORY MITES—A FOOD WEB APPROACH

**Ramana G. Colfer**
Department of Entomology and Center for Population Biology
University of California, Davis

**Goal:** To determine if augmentative releases of a predatory phytoseiid mite (*Galendromus occidentalis*) into the cotton agroecosystem can be used to control herbivorous mites, and to examine how naturally occurring generalist insect predators affect the success of augmentative biological control.

**Rationale:** Issues such as pesticide resistance, environmental degradation, water quality, and worker safety have promoted researchers, growers, and policy makers to pursue alternative strategies to pest management. Biological control has emerged as one of the more successful, safe, and sustainable strategies. This research will help to determine how ecological interactions between natural enemies influence the success of augmentative biological control and could advance augmentative biological control several steps closer to implementation by commercial agriculture.

**Approach:** A food web approach will be taken to determine whether predatory insects disrupt augmentative biological control by feeding on predatory mites or improve it by primarily feeding on herbivorous mites. This research will also evaluate the quality of commercially available predaceous mites and of a mechanical release device. During 1997 and 1998, field plot and field cage experiments will be conducted to test factors influencing the ability of predatory mites to suppress herbivorous mite populations. The factors to be tested include the following: (1) predatory mite release technique (manual *vs.* mechanical release device); (2) predatory mite release rate (5000 *vs.* 37500 *vs.* 250000 per hectare); (3) time of day of release (early morning *vs.* midday); (4) quality of the commercial source of the predatory mites; (4) plant developmental stage (cotton plant with 3-6 nodes *vs.* 8-12 nodes); and (5) presence of resident generalist insect predators.

Predatory mite releases in conventionally and organically managed cotton fields will continue to determine if such releases are feasible at larger scales and are compatible with a variety of agricultural management practices. Predaceous and herbivorous arthropod populations will be monitored using leaf and sweep net sampling techniques from the time of predatory mite releasee until cotton harvest. Observations of key arthropod species foraging behavior will be performed to construct a quantitative food web for the herbivorous mite subweb and to evaluate the determinants of food selection for generalist insect predators and the released predatory mite. Finally, the quality of predatory mites provided by various insectaries will be examined on the basis of viability, ratio of adults to juveniles, ratio of males to females, and the ability to establish in the field.

**Status:** During 1996, 36 predatory mite releases were performed in conventionally and organically managed grower cotton fields. Last year's research isolated factors that are likely to influence predatory mite release efficacy and established feasible experimental methods and sampling techniques.

# EFFECTS OF ENVIRONMENTAL CONTAMINANTS ON STEROID HORMONES IN THE AMERICAN ALLIGATOR

**D. Andrew Crain**
Department of Zoology
University of Florida

**Goal:** To examine circulating steroid hormones and gonadal steroid production in alligators exposed to environmental contaminants.

**Rationale:** Previous studies have documented endocrine disruption in American alligators exposed to environmental contaminants. This study examines the mechanisms through which these environmental contaminants exert their effects on the alligators by studying circulating hormone concentrations in wild alligators exposed to environmental contaminants and studying steroid production in alligators experimentally exposed to some of the environmental contaminants found in tissues of the wild alligators. It is hoped that this study will further elucidate endocrine disruption in the alligators and will provide evidence for a cause-effect relationship between specific environmental contaminants and the endocrine disruption.

**Approach:** Two studies were conducted to address this question:

*Study 1:* Blood samples were collected from juvenile alligators (60-140 cm) from three lakes in Florida. The lakes were chosen based on historical and ongoing episodes of exposure to environmental contaminants. Lake Woodruff (in Lake Woodruff National Wildlife Refuge) served as a reference lake, and Lake Apopka and Lake Okeechobee served as contaminated lakes. Blood was analyzed for circulating estradiol-17ß ($E_2$) and testosterone (T) concentrations. Whereas there were no differences in plasma $E_2$ concentrations among animals of the three lakes, male alligators from the contaminated lakes had significantly lower T concentrations compared to males from the reference lake.

*Study 2:* Uncontaminated eggs collected from the reference lake were exposed to several of the environmental contaminants previously measured in eggs from the contaminated site (Lake Apopka). Eggs were divided into five groups. Two of the treatment groups were controls (one group incubated at a male-producing temperature, and one group incubated at a female- producing temperature), one group was an endocrine-disrupting standard (estradiol-17ß), and two groups were common environmental contaminants (*p,p'*-DDE and *p,p'*-DDD, both metabolites of DDT). At hatching, gonadal production of $E_2$ and T was measured. Hatchlings treated *in ovo* with *p,p* '-DDD exhibited elevated T synthesis when compared to the other groups.

**Status:** The results of *Study 1* indicate that circulating levels of testosterone are reduced in animals from the contaminated lakes. This reduction does not appear to be due to altered gonadal hormone production, as *Study 2* indicates that T production is actually increased in DDD-exposed alligators. This suggests that endocrine disruption may occur at levels other than steroidogenesis. Future studies will explore steroid excretion rates of the liver and steroid-binding protein dynamics as potential points of disruption.

# EXPRESSION OF *OPH* (ORGANOPHOSPHORUS HYDROLASE) IN *TRICHODERMA VIRENS*

**Deanna Cross**
Department of Plant Pathology and Microbiology
Texas A&M University

**Goal:** To increase the efficiency of bioremediation of organophosphorus neurotoxins using the opd gene expressed in *T. virens.*

**Rationale:** Organophosphorus neurotoxins are xenobiotic (biologically foreign) compounds used as pesticides and chemical warfare agents. These compounds can be lethal at very small doses. Organophosphorus hydrolase *(oph)* has been shown to catalyze the hydrolysis of the P-O bond and to a lesser extent the P-F, P-S, and P-CN bond, making this enzyme an attractive component of organophosphate degradation. The potential for degradation is increased by placing this enzyme in a fungal system. For effective bioremediation to occur, it is imperative that the introduced gene be stable over a long period of time and that the produced enzyme be capable of interacting with the substrate. For these reasons, it is important to develop a stable transformation vector and a method for exporting the oph enzyme from the cell membrane into the substrate.

**Approach:** A homologous transformation system is being created using an arginine biosynthesis gene as the homologous integration site. Homologous transformation of DNA has been shown to be more stable in other systems than random integration events. A strain of *T. virens* that has a nonfunctional *argII* gene will be transformed with a vector containing the non-promoter portion of the *argII* gene and the *opd* gene driven by the *gpd* promoter, a constitutive *T. virens* promoter. Analysis of the transformants will be performed to determine the number of vector integrations per transformant. The transformants will then be assayed for stability and *oph* production and compared to transformants with a random integration pattern to discern the effects of homologous transformation on expression of the *opd* gene.

In addition to a homologous transformation system, a transformation vector that facilitates the export of *oph* is being tested. This transformation vector will contain the same portion of the *argII* gene along with the *opd* gene. However, the signal sequence *oph* will be removed and replaced with the signal sequence of *cbh* (cellobiohydrolase), a protein commonly exported from the cell. In addition to replacing the signal sequence, the promoter and terminator of the *cbh* gene will be used to drive expression of *opd.* The transformants will be assayed for production of *oph* and studies will be conducted to determine the amount of extracellular *oph* activity in the transformants.

**Status:** An *argII* mutant has been identified and *argII* has been placed in a vector. The *opd* gene is being incorporated to form a homologous integration vector. The region coding for the signal sequence and terminator region of *cbh* has been attached to the *opd* gene. The completion of the transformation vectors is expected shortly, and transformations should be complete by the end of the summer. Analysis should follow shortly thereafter.

# DIFFUSIONAL RATE LIMITATIONS TO CONTAMINANT TRANSPORT IN HETEROGENEOUS GROUNDWATER AQUIFERS

**Jeffrey A. Cunningham**
Department of Civil Engineering
Stanford University

**Goal:** To elucidate the impact of diffusional rate limitations upon contaminant transport behavior in saturated, heterogeneous, porous media (*i.e.*, groundwater aquifers), with a view to evaluating the role of such limitations in groundwater remediation. Specific objectives are: (1) to determine the range of spatial and temporal scales over which diffusional rate limitations can be manifested in natural environments; (2) to formulate criteria for evaluating the significance of small-scale diffusional rate limitations relative to other physical phenomena; and (3) to develop an efficient method for modeling contaminant fate and transport under the influence of diffusional rate limitations over a range of spatial and temporal scales.

**Rationale:** Due to the complex nature of the subsurface environment, aquifer restoration from contamination by hazardous chemicals is a surprisingly difficult and time-consuming endeavor. Slow release of contaminants from diffusionally accessed storage, whether in large-scale regions of low permeability or in small-scale intra-granular pores, is a crucial factor controlling the overall rate of remediation. This is true for innovative approaches like *in situ* bioremediation as well as conventional methods like pump-and-treat. By contributing to the fundamental understanding of the impact of heterogeneities at differing scales, this project will enable more accurate assessment of the time evolution of contaminant concentration in flowing groundwater. This research can be applied not only to the design and evaluation of remediation techniques but also to site characterization and the interpretation of field and laboratory data.

**Approach:** Analysis begins at the smallest scale (*i.e.*, individual grains of aquifer solids) and works up to progressively larger domains. The project is divided into four phases: (1) Develop a mathematical model that accounts for diffusion processes occurring on the scale of soil grains. Test the model by using it to describe the results of a set of laboratory experiments on contaminant removal from a sandy porous medium, the results of which suggest the presence of two distinct intra-granular diffusion processes. The model should account for all relevant processes at this scale. (2) Use the results of phase one to determine the range of temporal scales at which diffusion can occur on the grain scale. Use temporal moment analysis to evaluate the potential importance of grain-scale diffusion processes on contaminant transport in groundwater, paying particular attention to the way in which grain-scale processes can affect the movement of contaminant plumes. (3) Evaluate the significance of small-scale diffusional processes relative to larger-scale phenomena (*e.g.*, diffusion and slow advection through large regions of low permeability) and perform simulations of pump-and-treat and *in situ* remediation of computer-generated contaminated aquifers. At this stage, accurate representation of both small- and large-scale phenomena is critical to understanding the impact of aquifer heterogeneity across a spectrum of scales. (4) Use the results of the first three phases to interpret the results from actual field-scale transport experiments and remediation projects.

**Status:** Phases one and two as described above have been completed, and phase three has begun. To date, three manuscripts have been submitted for publication in refereed journals (currently under review), and an oral presentation was given at the 1996 Fall Meeting of the American Geophysical Union. An oral presentation will also be given at the 27th Congress of the International Association for Hydraulic Research (August 1997). Research will be complete in late 1997 or early 1998, with final thesis revisions completed no later than June 1, 1998.

# MOTIVATION AND EFFECTIVENESS OF VOLUNTARY POLLUTION CONTROL

**Lisa A. Damon**
Department of Agricultural and Consumer Economics
University of Illinois Urbana-Champaign

**Goal:** To identify factors that motivate firms to participate in voluntary pollution reduction programs, and to examine the impact of participation in such programs on subsequent pollution levels and future business performance.

**Rationale:** Environmental protection is a costly activity in our society. In recent years, EPA has established several voluntary programs to encourage efficient use of resources and promote pollution prevention. These programs rely on voluntary self-regulation rather than mandated command and control. If successful, these programs have the potential to control pollution while preserving the economic performance of business. A greater understanding of what motivates firms to join voluntary programs and how effective these programs are can help regulators develop more cost-effective environmental policy.

**Approach:** The factors that motivated firms in the chemical industry to participate in EPA's "33/50 Program" were examined. Panel data for 1988-1993 were used. Public firms with primary business activities in SIC Code 28 (chemical manufacturing) were identified. The Toxics Release Inventory (TRI) was used to identify the total toxic emissions of these firms and their releases of the 17 chemicals targeted in the 33/50 Program. Information about firm participation in the program was obtained from EPA. Of the 124 firms in the sample, 74 were participants in the program. Two additional sources provided other information for the study: The Standard & Poor's Compustat database provided firm-specific business characteristics such as number of employees, sales, and R&D expense. To add an indicator of potential Superfund liability of the firms under study, the Site Enforcement Tracking System (SETS) database was used to identify the number of PRP notifications each firm had received. Econometric techniques were used to identify the significant factors that led firms to participate in the program. Econometric models were also used to assess the difference in subsequent emissions between participants and non-participants. The impact of participation on the financial performance of these firms will be examined.

Status: This project is expected to be completed by May 1997. In June 1997, the preliminary examination in Environmental Economics will be written. The formal dissertation proposal will be developed after the examination is complete. The findings of this research will be used to help develop a topic.

# THE IMPORTANCE OF METHODOLOGICAL CHOICE IN THE VALUATION OF ENVIRONMENTAL GOODS AND SERVICES

**Jennifer Davis**
Department of Environmental Sciences & Engineering
The University of North Carolina at Chapel Hill

**Goal:** This research explores whether and how choice of data collection mode (in-person, by telephone, or through a focus group interview) influences the policy-relevant conclusions reached by investigators estimating the benefits of environmental goods or services.

**Rationale:** The contingent valuation method (CVM) has largely been adopted as the technique of choice for environmental benefit estimation. Members of the 1993 CVM expert panel convened by the National Atmospheric and Oceanic Administration (NOAA) included in their final recommendations the stipulation that information should be collected, whenever feasible, in private, face-to-face interviews with respondents. This research will contribute evidence regarding the ability of other less costly methodologies to produce equally reliable benefits estimates.

**Approach:** During May-July 1996, a willingness-to-pay study for improved water supply service was conducted in the city of Odessa, Ukraine. Four parallel data collection efforts were undertaken: an in-person household survey; an in-person intercept or "convenience" survey; a telephone survey; and twelve focus group interviews. The samples are non-overlapping (no Odessa resident participated in more than one data collection exercise). The data produced with these methods were not all collected concurrently but were collected within a ten-week period such that the economic, environmental, and political conditions of the city remained relatively constant throughout the study.

Participants in each data collection exercise were asked about their existing water supply service, including perceptions of their water quality in terms of taste, color, odor, and risk to human health. Individuals then received a description of the features of a modern water supply system and were asked their willingness to pay increased water tariffs to obtain such a system in Odessa. Socioeconomic and demographic data were also collected from each individual.

These samples will be analyzed independently to investigate whether significant differences in sample composition or policy conclusions arise from the conduct of each methodology under current "best practice" approaches. In addition, factor analysis will be employed to compare the complex relationships between socioeconomic, demographic, and attitudinal information and residents' willingness to pay for improved services among the four samples.

**Status:** All data needed for this analysis were collected during the period May-July 1996. Independent analysis of two of the four data sets (the in-person household and the intercept survey samples) has been completed, yielding interesting differences in sample composition but few significant differences in policy implications regarding the improvement of water service to residents. Analysis of the remaining data sets and completion of factor analysis described above are expected to be completed within the next nine months.

# MINERALIZATION OF BTEX MIXTURES BY ENRICHED AND PURE MICROBIAL CULTURES

**Rula Anselmo Deeb**
Department of Civil and Environmental Engineering
University of California at Berkeley

**Goals:** To trace the mineralization of the individual components of BTEX (benzene, toluene, ethyl benzene, xylenes) mixtures by enriched and pure cultures indigenous to a gasoline-contaminated aquifer, and to identify the key inhibitory and stimulatory processes governing the aerobic biotransformation of these mixtures.

**Rationale:** BTEX compounds are commonly used in the industry as degreasers, solvents, and gasoline constituents. These chemicals are often detected in the subsurface as a result of improper waste disposal, and have been classified by EPA as priority environmental pollutants due to their confirmed or suspected carcinogenity. Bioremediation has the potential to treat BTEX contaminated soil and water in a cost-effective and efficient manner. Because of the difficulties inherent in drawing direct conclusions about the mineralization of specific BTEX compounds in the field, controlled laboratory studies are needed to determine the key processes governing BTEX biological transformations. These processes can then be quantified and interpolated back to the field. Characterizing the influence of substrate interactions on the mineralization of BTEX mixtures will contribute to a better understanding of the fate of these hazardous organics in the subsurface. Knowledge gained in this study should therefore provide useful insights leading to new approaches to *in situ* bioremediation.

**Approach:** To date, no microorganism in the literature has been reported to mineralize a mixture containing all of the BTEX aromatics. As a result, some recent research in this field has focused on genetically constructing a bacterium that incorporates the requisite biological pathways. The central hypothesis for this research states that a mixed microbial consortium, indigenous to a gasoline-contaminated aquifer, will be capable of mineralizing complete mixtures of BTEX compounds. The sub-hypothesis states that pure cultures, isolated from this consortium, will exhibit different degradation capabilities towards BTEX mixtures and that the parent culture, as a result of syntropic relationships between its various microorganisms, will be better equipped to completely mineralize mixtures of BTEX. These hypotheses will be tested in detail through carefully designed laboratory experiments. Complete degradation of BTEX compounds and concurrent production of $CO_2$ will be monitored. Radio-labeled ($^{14}C$) BTEX will be used to trace compound mineralization. Kinetic studies will be performed to identify and quantify substrate interactions of BTEX mixtures using experimentally-determined degradation rates of BTEX in bisubstrate degradation studies.

**Status:** A mixed culture has been seeded from a gasoline contaminated aquifer and enriched in a continuous flow reactor at experimentally-determined optimal conditions using toluene as the sole carbon and energy source. Studies have shown this culture to mineralize all BTEX compounds individually and at high substrate concentrations. Studies with BTEX mixtures have revealed complete transformation of the parent BTEX compounds with production of $CO_2$. Since it is unclear whether complete mineralization of each of the BTEX components within the mixture took place, $^{14}C$-labeled BTEX components have been purchased and studies are currently underway to trace BTEX mineralization. Mixed culture studies have shown ethyl benzene to be a potent inhibitor of the biodegradation of benzene, toluene, and xylene mixtures; kinetic studies are in progress to quantify these inhibition effects. Two pure cultures *(Rhodococcus bronchialis* and *R. rhodochrous)* have been isolated from the mixed microbial consortium and are being tested for BTEX biodegradation capabilities.

# DEVELOPMENTAL METHYLMERCURY ADMINISTRATION ALTERS SYNAPTIC EXPRESSION OF NEURAL CELL ADHESION MOLECULES

**P. Markus Dey**
Joint Graduate Program in Toxicology
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**Goal:** To investigate the role of altered expression of neural cell adhesion molecules (NCAM) in metal-induced synaptic injury by integrating mechanistic and behavioral toxicology in the assessment of developmental neurotoxicity. Preliminary data indicate that metals disturb NCAM expression, but data directly linking vulnerability of NCAM to metals during critical stages of development are incomplete.

**Rationale:** Environmental exposure to heavy metals during development remains a pressing public health issue in the United States. Of particular concern is developmental exposure to the ubiquitous toxic metal methylmercury (MeHg). Exposure to this toxicant during brain morphogenesis can result in brain malformations. More common, however, are low-level exposures that result in quantitative reductions of IQ and subtle psychomotor retardation without causing overt signs of maternal toxicity. The lack of mechanistic understanding of these effects has delayed development of strategies to prevent or ameliorate low-level toxic effects. It is now possible to examine the impact of toxic chemicals on specific molecules whose critical roles in brain development and learning and memory have been characterized.

**Approach:** Morphological, biochemical, and behavioral studies are designed to correlate altered NCAM expression, retardation or loss of synaptic structure, and behavioral deficits. The effects on NCAM are assessed in cerebellum, hippocampus, and forebrain of rat pups exposed to MeHg during early postnatal life (day 3-15) and sacrificed on days 15, 30, and 60. Research techniques localize these antigens to specific brain regions and synaptic sites. Electron microscopy documents altered synaptic structure following MeHg treatments. Preliminary data indicate that MeHg perturbs post-translational modification of NCAM. Activity of golgi sialyltransferase (ST), an enzyme crucial to NCAM modifications during development and in learning acquisition and consolidation, will be measured following *in vivo* and *in vitro* MeHg exposure. Correlation between morphological/biochemical NCAM data and behavioral studies will identify possible mechanisms underlying learning disturbances following exposure to MeHg.

**Status:** Substantial progress has been made in ascertaining biochemical and morphological changes of NCAM expression following MeHg exposure. Age- and region-specific alterations of brain NCAM expression have been demonstrated. Increased ST activities are observed both *in vivo* and *in vitro,* indicating that MeHg alters NCAM expression by modulating the ST enzyme. Research indicates that changes of hippocampal NCAM persist into adulthood and may be related to the learning and memory deficits observed by other investigators following low-level MeHg exposures. It is anticipated that this research will be completed by the spring of 1998.

# LIGHT ABSORPTION BY LABORATORY AND AMBIENT ELEMENTAL CARBON PARTICLES

**Ann M. Dillner**
Civil Engineering Department
University of Illinois

**Goal:** To test the hypothesis that mass absorption efficiency of elemental carbon may be more a function of size distribution than of location by measuring absorption efficiency as a function of particle size and wavelength of light.

**Rationale:** Atmospheric aerosols affect global climate by scattering and absorbing solar radiation, thereby altering the temperature profile in the atmosphere. Particles which scatter light cool the atmosphere while particles which absorb light warm the atmosphere. Elemental carbon, produced mainly from incomplete combustion of fossil fuels, is the primary absorber in the atmosphere. Light absorption measurements, integrated over all sizes of particles, indicate different absorption efficiencies at different locations. By studying the light absorption rates of elemental carbon as a function of particle size, the climate effects due to the particles can be determined if the elemental carbon particle size distribution is measured.

**Approach:** Laboratory and atmospheric experiments will be performed to determine the mass absorption efficiency (the absorption coefficient divided by the concentration of particles in the sampled air) of elemental carbon as a function of size distribution and wavelength. Monodisperse laboratory elemental carbon samples and eight size ranges of polydisperse laboratory particles and ambient particles will be collected. The mass of particles collected and the flow rate of the sampling will be measured to calculate the concentration of particles in the sampled air. The absorption coefficient of the particles will be determined using two techniques. In both cases, light scattered by the particles will be reflected off the internal surfaces of the equipment and collected by the detector so that the attenuation of the light is due solely to absorption. The difference between the light transmission with and without particles establishes the absorption coefficient. Ambient and laboratory absorption efficiencies will also be modeled with the Mie code, the exact mathematical representation of light incident on spherical particles. We hypothesize that measured and modeled size-dependent absorption efficiencies will agree, indicating that ambient light absorption efficiencies can be calculated from known elemental carbon size distributions and that elemental carbon masses may be determined through filter-based absorption measurements.

**Status:** Research has just begun. Ambient sampling is planned for July and August of this year, and work is expected to be completed within two years.

# CHEMICAL AND MICROBIOLOGICAL TREATMENT OF ACID MINE DRAINAGE FOR THE REMOVAL OF IRON AND ACIDITY

**Harry R. Diz**
Charles E. Via Department of Civil Engineering
Virginia Polytechnic Institute and State University

**Goal:** To investigate the feasibility of a new technology for the treatment of acid mine drainage from active and abandoned mine sites that would accomplish iron removal without the need for large settling ponds and without formation of iron hydroxide sludge.

**Rationale:** Acid mine drainage (AMD) continues to be an important water pollution problem in the United States and around the world. The primary characteristics of AMD which cause concern are low pH (undiluted seeps typically pH 2.0-3.0), high specific conductivity, high concentrations of iron, aluminum, and manganese, and the variable presence of toxic heavy metals at trace levels. Water quality and habitat are severely degraded by such inputs, and aquatic biodiversity is impacted, sometimes with the total elimination of higher life forms. Current treatment technologies are either inadequate or too costly to be employed at the numerous abandoned mine sites containing untreated AMD. Limestone employed for pH adjustment quickly becomes coated with iron oxyhydroxide, drastically reducing its solubility. Constructed wetlands have been relatively successful in locations where topography and space are available but result in the creation of metal-contaminated soils and groundwater.

**Approach:** The research investigates the fundamental chemistry and microbiology of ferrous and ferric sulfate solutions typical of acid mine drainage settings. It has been noted by many observers that iron forms oxide coatings in AMD environments. Therefore, it was hypothesized by the current investigator that this tendency to form coatings could be optimized in an engineered setting. Since ferric iron is increasingly insoluble above pH 4, low iron effluent concentrations could be achieved without the formation of iron hydroxide sludge if iron could be precipitated as a coating rather than allowed to spontaneously nucleate, thus requiring a sedimentation basin for removal. The first step was to employ microorganisms to oxidize ferrous iron to the ferric state while maintaining the iron in a dissolved state. Abiotic iron oxidation is not useful since it proceeds very slowly until pH is increased above pH 5, at which point spontaneous nucleation occurs instantaneously. Thus, the research includes studies of the oxidation kinetics of iron-oxidizing acidophilic bacteria, which produce dissolved ferric iron at low pH (<2.5). These naturally occurring bacteria require no artificial food source since they gain energy from iron and carbon from $CO_2$. It was observed that, upon the addition of less than the stoichiometric requirement of base to a ferric sulfate: solution, a time lag occurred before the appearance of a precipitate. It was hypothesized that, during this induction period, the growing iron polymers would be more likely attach to and become part of a previously formed oxide surface than to form new nuclei, due to a lower activation energy barrier. The factors affecting this behavior were investigated and the phenomenon was documented.

**Status:** Better than 90% removals have been achieved while producing a clear effluent. The experimental work is expected to be completed by the end of March, 1997. Pilot-scale testing of the technology would be the next step in the development process but is beyond the scope of the current doctoral research program.

# PREDICTING CLIMATE CHANGE AND ECOSYSTEM INTERACTIONS: SYNTHESIS OF MULTIPLE FIELD METHODS

**Jennifer A. Dunne**
Energy and Resources Group
University of California, Berkeley

**Goal:** To compare and synthesize results from an ongoing ecosystem warming experiment with measurements of vegetation, nutrient, and microclimate dynamics along a natural climatic gradient and within a new set of warming-related climate manipulations along the gradient. This research is focusing on interactions of climate warming and vegetation structure and dynamics within an ecotone of subalpine meadow and Great Basin shrub steppe.

**Rationale:** In trying to predict the impacts of climate change on ecosystems and feedbacks from ecosystems to climate change, scientists face several methodological dilemmas. First, none of the approaches to investigating climate change and ecosystem interactions (laboratory experiments, microcosm experiments, field experiments, observations along natural climatic gradients, palynological studies, simulation modeling) provide direct investigations of climate change at the rate and magnitude of concern, due to rapid forcing by doubled $CO_2$. Second, each approach has well-known drawbacks. Third, within each approach, many particular methods can be utilized. The most useful strategies will integrate results from multiple approaches and methods.

**Approach:** The current research is being conducted in montane habitat within large patches of mixed subalpine meadow/shrub-steppe vegetation in the upper East River Valley of Gunnison County, CO. One site (the "warming meadow") was established by Professor John Harte at the Rocky Mountain Biological Laboratory in 1991 and has a set of five control plots and five manipulated plots with overhead heaters that increase downward IR flux as predicted under doubled $CO_2$. Three additional sites were established in 1995 along an elevation gradient centered near the warming meadow. Each of the new sites has five control plots and five manipulated plots in which snow melt is accelerated via shoveling and summer soil moisture is reduced via rain diversions. These two effects are the strongest climatic impacts of the ongoing warming experiment and are predicted to be the strongest climatic impacts of anthropogenic climate change in this region. Timing of snow melt differs naturally among the gradient sites to the same degree that it differs between control and manipulated plots at any one site (about 1-2 weeks).

In each of the plots, dynamics of the following parameters during the growing season are monitored: plant reproductive phenology; aboveground biomass in shrubs, forbs, and graminoids; plant community composition; seedling establishment; shoot elongation; leaf photosynthetic rates; soil inorganic nitrogen stocks; and soil nitrogen net mineralization rates. Aspects of the carbon cycle, plant albedo (reflective power), soil moisture and temperature, timing of snowmelt, hydrology, and weather are also monitored.

**Status:** In 1996, snow shoveling manipulation was initiated, soil microclimate data logging systems were installed, and monitoring of some of the parameters detailed above was begun. The summer of 1997 marks the first season of complete data collection with full implementation of combined snow-plus-soil-moisture climate manipulation techniques. At least three seasons of data will be collected to incorporate annual climate variation and delayed and cumulative effects into analyses.

# RP4 PLASMID TRANSFER AMONG STRAINS OF *PSEUDOMONAS* IN A BIOFILM REACTOR

**Laura J. Ehlers**
Department of Geography and Environmental Engineering
The Johns Hopkins University

**Goal:** To observe and quantify conjugation between bacteria growing in a biofilm reactor.

**Rationale:** The horizontal exchange of genetic material between bacteria is a fundamental activity of natural microbial populations, responsible for the wide-spread dissemination of antibiotic resistance among bacteria and the development of synergistic bacterial/plant interactions. The most prevalent mechanism of transfer, conjugation, is mediated by plasmids, extrachromosomal DNA elements that are critical to the development of genetically engineered microorganisms (GEMs) for use in environmental, agricultural, and biotechnological applications. Common usage is hampered by incomplete knowledge of the fate of GEMs upon release into the environment, including the potential for their plasmids to transfer into indigenous bacteria. Risk assessment guidelines cite the need for quantitative rates of plasmid transfer in the environment. Over 90% of bacteria in nature are attached to surfaces as biofilms. However, very few conjugation studies have been conducted employing Systems representative of natural biofilms. Previous studies suggest that conjugation rates on surfaces may be elevated compared to rates in liquid media.

**Approach:** A biofilm reactor was chosen for its experimental flexibility and simple mathematical description. Several environmental parameters within the reactor were varied to determine their influence on transfer frequencies. Finally, a predictive biofilm model was evaluated for its ability to accurately represent plasmid transfer events.

Conjugation of the broad host range plasmid RP4 between two strains of *Pseudomonas* was found to occur in the biofilm reactor at high frequencies. The most important environmental parameter was the shear stress at the biofilm-liquid interface. Increasing temperature from 150°C to 28°C increased conjugation frequencies 10,000-fold. Conjugation frequency was unchanged in experiments conducted with 3.5, 7, and 35 mg/l acetate, although total cell concentration in the biofilm increased as expected. Finally, the addition of a sublethal kanamycin concentration affected both the recipient and transconjugant populations, either by enhancing conjugation or providing a selective advantage for transconjugant growth. These data suggest ways to manipulate environmental parameters to increase or decrease plasmid transfer rates among biofilm bacteria.

**Status:** The bulk of the experimental data has been collected and analysis is set to begin.

# USE OF SINTERED GLASS AS A MEDIUM IN INTERMITTENTLY LOADED FILTERS: FATE OF VIRUS

**Robert W. Emerick**
Department of Civil and Environmental Engineering
University of California at Davis

**Goal:** To demonstrate that virus removal can be achieved in small wastewater treatment systems by designing a biological system engineered for optimal removal and suitable for use in residences and small communities.

**Rationale:** Viruses are a wastewater contaminant that can be removed. Microorganisms have been linked with viral degradation in natural systems. Therefore, the potential exists for the biodegradation of viruses. However, no engineered biological system has been developed to date specifically for removal of virus from wastewater.

**Approach:** The optimal design and operation of intermittent sand filters (ISFs) to remove organic material and nutrients using sand as a filtering medium is well documented. It is hypothesized that the current design criteria would have to be altered for virus removal, because degradation of the virus coat protein would be expected to be a more substantial undertaking, unlike biological degradation of organic wastes, which does not require an extensive biological population.

Sintered glass (effective size = 1.5 mm) was chosen as a filter medium because of its large surface area (87,056 $m^2/m^3$) and large effective size. A large effective size is desirable because it encourages aerobic conditions deep within the filter and also prevents the formation of a biological mat that could block the pore spaces and result in filter clogging. Laboratory-scale filter columns filled with sintered glass medium are being investigated as a means of wastewater treatment specifically for the removal of virus. The filters are loaded intermittently with primary effluent at a hydraulic loading rate equal to the field capacity of the filter each day, with dosing frequencies of 1, 2, 3, 12, 24, and 48 times/day. MS2 coliphage is being used as a model virus because its size and physical characteristics are similar to human enteric viruses, and it is among the most resistant of all viruses to environmental degradation.

**Status:** Testing is complete utilizing a dosing frequency of 1, 2, and 3 doses/day. Testing with dosing frequencies of 12, 4, and 48 times/day will begin April 1 and be complete by the end of June. A paper is anticipated to be complete by the end of August, 1997. Results to date indicate that frequent dosing results in virus removal from the effluent that is 3-6 times more effective than low dosing frequencies.

# INTRA-POPULATION SEX RATIO VARIATION IN THE SALT GRASS *DISTICHLIS SPICA TA.*

**Sarah M. Eppley**
Center for Population Biology
University of California, Davis

**Goal:** To determine the proximate and ultimate mechanisms responsible for spatial segregation of the sexes (SSS—*i.e.*, males and females living in different microhabitats), in the salt marsh grass, *Distichlis spicata.*

**Rationale:** In order to preserve and restore wetland areas, information on wetland ecosystems is required, particularly the factors that make them resistant to degradation and resilient to change. Genetic structure of populations can greatly influence their stability and consequently the stability of the ecosystem of which they are a part, yet little is known about the genetic structure of wetland plant populations and how such structure changes over time. One major component of genetic structure in many wetland plant species, spatial segregation of the sexes, is often totally ignored. Such segregation is difficult to explain evolutionarily as well as ecologically. Except in species with environmental sex determination, even the proximate mechanisms for the pattern have not been determined for the more than 18 primarily wetland species for which the pattern has been documented.

**Approach:** The proximate mechanisms responsible for SSS in *D. spicata* will first be determined and then selection pressures acting to maintain such a pattern will be addressed. SSS is generally described by researchers counting reproductive ramets (ecologically, not necessarily genetically, distinct individuals). Thus, possible proximate mechanisms include clonal spread of a few individuals; environmental sex determination; differential flowering of males and females in different patches; differential growth of clones; differential germination requirements; and differential mortality. At three sites along the northern California coast, field experiments combined with RAPD-PCR markers capable of differentiating clones and determining the sex of individuals will be used to test these mechanisms. At a fourth site at Mono Lake California, populations of different known ages will be compared in order to determine how SSS and genetic structure in general change through time in this species.

**Status:** RAPD markers which can differentiate clones have been identified, and a marker which consistently co-segregates with female phenotype has been found. These markers on samples from three coastal field sites have been used to rule out clonal spread of a few individuals, environmental sex determination, differential flowering, and differential clonal growth as proximate mechanisms for SSS in these populations. Seeds and seedlings have been planted in the field in order to test differential germination requirements and differential mortality between the sexes. A two-year reciprocal transplant study is being monitored and pollen augmentation data are being analyzed for selection pressures. RAPD data taken from samples from Mono Lake are also being analyzed to look at changes in genetic structure through time, and a computer simulation model has been developed to aid in understanding experimental results.

# FACTORS INFLUENCING COMMUNITY DEVELOPMENT IN PROTECTED AREAS OF THE NEW TROPICS: A POLITICAL ECOLOGY OF CUYABENO WILDLIFE RESERVE, ECUADOR

**Juliet Serenyi Erazo**
College of Forest Resources
University of Washington

**Goal:** To analyze the political ecology of protected areas and their environs in the Ecuadorian Amazon, with the aim of identifying the government policies and local conditions that lead to management schemes supporting the dual goals of improved living conditions for rain-forest peoples and maintenance of rainforest ecosystem integrity.

**Rationale:** Governments and NGOs (non-governmental organizations) throughout the tropics are struggling to realize the seemingly conflicting goals of development and environmental sustainability. The high level of biological and cultural diversity in the tropics is being lost to a population that is increasingly exposed to homogenizing market forces. Protected areas provide a unique laboratory for experimenting with and identifying management schemes that respect both human dignity and species diversity.

**Approach:** Research conducted in 1994-1995 and during the winter of 1997 revealed that communities sharing the same ecosystem have radically different experiences with eco-tourism and other development projects favored by those interested in conserving natural areas. This research hypothesizes that these differences are due to differences is cultural traditions rather than governmental policies, but that governmental policies can be used to balance community tendencies towards nepotism and resource exploitation which threaten environmental conservation. During the months of January, February, and March of this year, interviews were conducted with community members living in and near the Cuyabeno Wildlife Reserve, located in northeastern Ecuador. Numerous interviews were also conducted with personnel from government agencies, development organizations, environmental groups, and tour operators working in the Reserve. Finally, ethnographic information on the communities was gathered from studies conducted over the last twenty years.

**Status:** As of early March 1997, field research in Ecuador is nearly complete, and the next three months will be spent analyzing findings and writing papers for publication.

# THE EFFECT OF CLOUDS AND AEROSOLS ON THE WAVELENGTH DEPENDENCE OF ULTRAVIOLET AND VISIBLE SOLAR RADIATION AT THE EARTH'S SURFACE

**Carynelisa Erlick**
Department of Geophysical Sciences
University of Chicago

**Goal** :To model the spectrum of solar radiation at the Earth's surface at a "typical" continental or urban site with the aim of distinguishing the effects clouds and aerosols have on this spectrum and determining which mechanisms dominate its shape.

**Rationale**: The effects of clouds and aerosols on solar radiation have challenged radiation modelers, climatologists, and atmospheric chemists alike. Relatively recently, climatologists have turned to the study of aerosols to help explain the solar cloud forcing anomaly and the clear sky flux anomaly, namely that models are predicting more solar radiation reaching the ground than is indicated by measurements. This study is designed to improve the input to—and design of—such models so that they may give a more realistic measure of the amount of biologically damaging ultraviolet radiation reaching the ground in the more populous regions of the Earth.

**Approach**: As a first step in this project, a new solution to the radiative transfer equation for a plane parallel scattering atmosphere was formulated. Next, a set of "typical" microphysical parameters was extracted from the literature on atmospheric trace species, clouds, and aerosols to describe the following: nonabsorbing molecules; ozone in the stratosphere; absorbing and non-absorbing dry aerosols; the growth of aerosols with humidity; pure water cloud drops; ice within the upper layers of clouds; external mixtures of aerosols and cloud drops; inactivated aerosols and the partial activation of accumulation mode aerosols within cloud layers; the coagulation of soot particles with cloud drops and sulfate aerosols; dissolved carbonaceous absorbers inside cloud drops and sulfate aerosols; and gaseous pollutants in the lower troposphere, such as ozone, nitrogen dioxide, and sulfur dioxide. The final step of the project is to use these microphysical parameters as input to the model and use it to explore the importance of and competition between each of the above effects.

**Status**: Results so far indicate that the ways in which clouds and aerosols affect atmospheric transmission are quite different. Plans are to continue modeling various scenarios of cloud layers superimposed on aerosol profiles, and to confirm modeled trends by comparing them with two sets of spectro-radiometer measurements, each spanning cloudy *vs.* clear and polluted *vs.* non-polluted atmospheric conditions. Research is expected to be completed by August 1997.

# GIS ANALYSIS OF HARVEST HISTORY

**Alexander Evans**
Yale School of Forestry and Environmental Studies
Yale University

**Goal:** To create a geographic database on the Yale-Myers Forests and answer the question of how tree cover has changed as a result of harvests.

**Rationale:** Yale-Myers has a long history of careful silvicultural management which could serve as an excellent information resource to both the forestry community and the American public. Yale-Myers could supply long term ecological data to be used as baseline data for impact assessments and other environmental studies. A spatial analysis of tree cover change is essential to gathering such data due to the importance to the ecosystem of geographical characteristics such as cut size or proximity to water courses. Yale-Myers is particularly suited to long-term investigation of spatial vegetation patterns because of its cartographic history and careful management.

**Approach:** The Yale-Myers mapping project has two elements: (1) Construction of an online, graphic database of forestry information about Yale-Myers designed for easy public access, and (2) Use of satellite images, in conjunction with a geographical information system (GIS) to investigate how current conditions differ from historical records and how current patterns could have been generated by past harvests. The platform for the Yale-Myers graphic database will be the world wide web (WWW) section of the Internet. By making use of the WWW, information will be widely accessible to the forestry community and interested public. The WWW bridges compatibility gaps which can hinder information flow, and computer search capabilities will allow a closer fit between information and user needs.

ERMapper software will be used to process a LANDSAT Thematic Mapper image of the Yale-Myers Forest area. The resulting image will show the current areas of forest cover and a measure of the near infrared emission of the vegetation, a good proxy for vegetation vigor. The processed image will be brought into a GIS (ArcView 3.0 with the Spatial Analyst extension) for analysis of spatial patterns and comparison with previous maps of the area.

**Status:** The skeleton of the public access website has been built and almost all current information on the Yale Myers forest has been digitized in ArcView 3. Remote sensing data have been collected but not yet analyzed. Remote sensing and spatial analysis will be finish by early May. The website will be fully operational by the end of May. The prototype website is accessible at http://www.yale.edu/schoolforest

# DYNAMIC PHOTOPROTECTION IN MARINE MICROALGAE

**Terence J. Evens**
Department of Ecology, Evolution and Marine Biology
University of California, Santa Barbara

**Goal:** To examine the dynamic response of marine microalgae to high light fluxes, specifically, the effects of static and dynamic light intensities/spectral distributions upon the synthesis and photoprotective functionality of xanthophyll-cycle pigments.

**Rationale:** Regulation of excess photon capture is undoubtedly the primary process that determines the *in vivo* quantum yield of photosynthesis under physiological conditions. It is hoped that this research will improve understanding of the effects of dynamic light regimes upon marine primary productivity. This should enable derivation of more accurate estimates of water column productivity than those based on static, *in situ* incubations.

**Approach:** The protocol for this research involves two main approaches: 1) Determine how various light intensities/spectral distributions effect photoprotective pigment pool partitioning within the chloroplast. The hypothesis is that a reciprocity between light intensity and spectral distribution determines the size and location of photoprotective pools. Two questions to be addressed are: (a) With regard to sizes of membrane-bound pigment pools, are all of the membrane bound xanthophyl 1-cycle pigments available for photoprotection? and (b) Are there species-specific responses to light field characteristics? 2) Compare and contrast the effects of dynamic *vs.* static light fields on photoprotective functionality within an intensity/spectral distribution framework. The hypothesis is that an oscillating, spectrally-dynamic light field, as opposed to a "traditional," static light field, produces a photoprotective functionality that is unique to that system. Questions to be addressed are: (a) How does this differ from the functionality of the photoprotective system derived from a static intensity/spectral distribution system? (b) Accessory pigment precursors or triplet oxygen scavengers: What possible functions can the non-membrane bound (or water soluble) pigment pools serve? (c) Are there species-specific functionalities? and (d) Does the difference in the two systems warrant the use of a dynamic model in determining productivity estimates from water samples incubated *in situ* or is it possible to arrive at a reasonable estimate of water column productivity through integration of fixed depth determinations?

**Status:** Presently, determinations of static light field and pigment pool partitioning and functionality are being made. This analysis is scheduled for completion by Fall 1997, and the dynamic light field experiments are slated to begin by January 1998. Research is expected to be complete by early 1999.

# CONTRASTING PATTERNS OF COMMUNITY RESPONSES TO ENVIRONMENTAL STRESSORS: THE ROLE OF ECOLOGICAL HISTORY AND COMMUNITY STRUCTURE

**Janet M. Fischer**
Center for Limnology, Department of Zoology
University of Wisconsin

**Goal:** To examine the generality of community responses to stress and explore the factors that determine community sensitivity to stress by considering whether different communities respond similarly to the same stress, and what roles ecological history and community structure have in controlling community sensitivity to stress.

**Rationale:** When community sensitivity to environmental stressors varies among systems, identification of sensitive systems can be a critical aspect of environmental protection. Assuming that species composition and ecological history are important determinants of community sensitivity, this information can be used to guide proactive protection measures.

**Approach:** A comparative experimental approach, in which identical replicated experiments were conducted in several systems simultaneously, was used to examine variation in zooplankton community responses to acidification. The three study sites differed in ecological history and community structure. In Trout Lake, pH is relatively high (7.9) and stable throughout the year. In contrast, pH is lower (6.1) in Little Rock Lake Reference Basin and the lake experiences a seasonal low pH near 5.2 during spring mixis. Little Rock Lake Treatment Basin was experimentally acidified to pH 4.7 during the late 1980s. When this experiment was conducted in 1995, the pH in Little Rock Lake Treatment Basin had recovered to 5.8.

Zooplankton community structure also differed among study lakes. For example, Trout Lake contained some species which are typically absent in more acidic lakes. In each lake, six experimental chambers (mesocosms) constructed of clear polyethylene cylinders sealed at the bottom and suspended from wooden rafts were deployed. Mesocosms were filled with lake water and stocked with zooplankton at approximately lake density. After a week of acclimation and collection of samples representing initial conditions, three replicate mesocosms were acidified to a target pH of 4.7 with dilute sulfuric acid. The remaining three replicates served as unacidified controls. Crustacean zooplankton were sampled weekly. For each lake, responses of the dominant zooplankton species were analyzed using Univariate Repeated Measures Analysis of Variance.

Results indicate substantial variation in zooplankton community sensitivity to acidification among study lakes. Zooplankton populations responded most strongly to acidification in Trout Lake. Zooplankton responses to acidification were less dramatic in Little Rock Lake Reference Basin and weakest in Little Rock Lake Treatment Basin. Several factors may have played a role in these contrasting patterns of zooplankton sensitivity to acidification: (1) differences in the absolute magnitude of pH change; (2) differences in species composition; and (3) interlake variation in acid tolerance arising from differences in ecological history.

**Status:** Laboratory bioassays are planned for this summer to examine interlake variation in acid tolerance for zooplankton species that occur in all three lakes. If acid tolerance differs among lakes such that sensitivity is greatest in Trout Lake and lowest in Little Rock Lake Treatment Basin, this result will suggest that communities may be more resistant to repeated stresses than to novel stresses. Research is expected to be complete by December 1997.

# MOLECULAR CHARACTERIZATION OF MARINE METAL-PRECIPITATING BACTERIAL SPORES

**Chris Francis**
Scripps Institution of Oceanography
University of California, San Diego

**Goal:** To elucidate and possibly enhance the metal-binding and precipitation properties of a marine bacterium for application in the removal of metal pollutants from the environment.

**Rationale:** Metal pollutants pose a significant problem because, unlike organic pollutants, they cannot be degraded. Instead, metals may be transformed into different forms (soluble vs. particulate), of which soluble metal ions are the most toxic. Thus, microorganisms that catalyze metal transformations influence both the toxicity and mobility of metals in the environment. The dormant spores of the marine manganese-oxidizing bacterium, *Bacillus* sp. strain SG-1, interact with a variety of metals, some of which (*i.e.*, Cd, Zn, Ni, and Cu) are bound to the spore surface while others (*i.e.*, Mn and Co) are both bound and oxidized. In addition, the metal oxides which form on the spore surface are capable of adsorbing and oxidizing many metals, radionuclides, and organic compounds. These unique features, combined with the highly resistant nature of bacterial spores, make SG-1 spores an attractive candidate for bioremediation applications. Also, since Mn oxidation is protein-catalyzed on the spore surface, molecular techniques can be used to study and possibly further enhance this process.

**Approach:** A cluster of seven genes involved in manganese oxidation by SG-1 spores, the *mnx* genes, were previously identified in our laboratory. One of these genes, *mnxG,* is the prime candidate for encoding the Mn oxidase. The 138 kDa gene product, MnxO, is related to the family of multicopper oxidases, a diverse group of enzymes that use multiple copper ions to oxidize a variety of substrates. The biochemical characterization of this key protein is essential for determining its precise role in Mn oxidation, as well as for assessing the potential for further enhancing the Mn-oxidizing ability of SG-1 spores. Polyclonal antibodies to Mnx0 have been cloned, expressed, and generated. Mnx0 has been localized to the outer layer of SG-1 spores. It has recently been discovered that this outer layer, termed an exosporium, can be removed by mechanical shear and still retain metal-oxidizing activity, demonstrating the potential for a "cell- free" metal removal system.

Current studies are focused on constructing plasmids containing the *mnxG* gene, introducing them into our non-oxidizing transposon mutants, and screening the spores for restored Mn oxidation. If this approach is successful, future studies will involve attempting to generate spores with hyperinduced expression of Mn-oxidation activity. This might be accomplished by modifying the proportion of different spore surface proteins, modifying the actual protein structures, or over expressing the Mn oxidase itself. Mutants can be readily screened for hyperexpression by the black colored oxides that typically precipitate on colonies grown on agar plates containing manganese.

**Status:** The research progress made thus far has focused on characterizing the key genes and proteins involved in metal-binding and oxidation by SG-1 spores. The next phase of this project, to be completed in the next year or so, will focus more on attempting to engineer this organism to make the spores (and exosporium) even more useful for metal removal processes.

# AMBIENT AIR POLLUTION AND DAILY MORTALITY IN NORTH CENTRAL TEXAS, 1990-1994: A TIME SERIES ANALYSIS

**Janet L. Gamble**
Department of Social Sciences
The University of Texas at Dallas

**Goal:** To determine whether: (1) significant, positive relationships exist between fine particulate matter ($PM_{10}$) or ozone and daily non-accidental death; (2) any observed pollution-mortality effects persist when weather, season, and other temporal factors are controlled; or (3) differential effects are observed either by cause of death or age at the time of death, by examining relationships between variations in ambient air pollutants and daily counts of death in Dallas County, Texas during the 1990-1994 period.

**Rationale:** Recent analyses have documented significant mortality effects associated with particulate matter, and to a lesser extent, other criteria pollutants. These effects have been observed at levels below the NAAQS. The majority of sites analyzed have been located along the east or west coasts of the U.S. or abroad. Pollution in the Dallas area is largely summertime ozone smog associated with automobile emissions rather than with coal burning or industrial output. Particulate matter concentrations remain well below federal standards. As such, Dallas offers a suitable site for assessing lower bound influences of particulate matter on mortality and further analyzing mortality effects related to ozone.

**Approach:** A time series of daily measures of criteria pollutants and aggregate non-accidental deaths were analyzed. Poisson regression models allowing for over dispersion are utilized to assess associations between the pollutants (estimated $PM_{10}$, ozone, and nitrogen dioxide) and daily counts of death. Deaths due to respiratory and cardiovascular causes are evaluated in separate analyses, as are deaths by age cohort. Seasonal effects and other temporal trends, including viral outbreaks, as well as variations in temperature and humidity, were controlled.

No association for any cause of death nor for any age group was found for particulate matter or nitrogen dioxide. However an increased risk of premature death was found to be associated with ozone. The relative risk of mortality associated with a 100 part per billion increase in concurrent day maximum ozone was 1.094 (95% CI = 1.012-1.183). For persons aged 70 and older the relative risk was slightly higher, 1.127 (95% CI = 1.022 - 1.244). Two-day lagged mean ozone was also significantly associated with mortality. These results refute prior studies that have suggested no lower bound threshold exists for an acute mortality effect of particulate matter. These results add to the handful of studies that find a significant ozone-related mortality effect.

**Status:** This analysis has constituted dissertation research, which is now complete. Oral defense is scheduled for April.

# BIASING RHIZOSPHERE ECOLOGY: THE IMPACT OF NUTRITIONAL MEDIATORS ON BACTERIAL POPULATIONS

**Brian B. McSpadden Gardener**
Department of Botany and Plant Pathology
Michigan State University

**Goal:** To evaluate the potential of selectively enhancing the growth and activity of inoculant microorganisms in the soil and rhizosphere environments.

**Rationale:** Microbial inoculants have a tremendous potential for enhancing agricultural productivity. However, the efficacy of microbial inoculants has been shown to be variable in a number of cases. To improve the reliability of microbial inoculants, it has been proposed that selective niche creation be used to enhance the activities of specific populations of bacteria. Several nutritional mediators, including opines, rhizopines, and salicylic acid, have been used to study the feasibility of specific niche creation. In soil and rhizosphere environments, the ability to catabolize these compounds appears to be rare, making them good candidates for selective niche creation.

**Approach:** In this study, rhizopines and closely related compounds are being used to assess the potential of enhancing inoculant populations and to evaluate the impact of such compounds on indigenous soil and rhizosphere communities. The methodology involves: (1) Quantification of indigenous catabolic activity by measuring growth on the substrates as sole carbon source and catabolism in complex mixtures. Using serial dilutions, the catabolic potential of the soil and rhizosphere environments are assayed over time. (2) Characterization of bacteria capable of catabolizing the selective substrates. Growth of the bacteria on the selective substrates in culture is compared to predict the relative competitiveness of inoculant and indigenous strains. (3) Evaluation of the benefit conferred to an inoculated rhizobium *(R. meliloti* 1021) relative to an otherwise isogenic mutant (1021[TnS::*idh*]) incapable of utilizing the selective substrate for growth. Under controlled conditions, germinating seeds receive daily nutrient amendment and samples are taken over a time course to investigate the dynamics of this amendment on bacterial populations. (4) Evaluation of the impact of nutrient amendment on the abundance and diversity of bacterial communities.

**Status:** To date, work on (1) and (2) described above has been largely completed. The catabolic activity of indigenous microorganisms has been assayed and used to determine the specificity of potential nutritional mediators. A number of rhizopine catabolizing strains have been isolated and characterized. The competitive ability of several of these isolates has been compared to *R. meliloti* L5-30 (the originally proposed inoculant), and they have been found to be superior competitors. Alternative inoculant organisms have been chosen for further study. Currently, the response of various bacterial populations to selective nutrient amendment is being characterized. The competition studies using 1021 will be completed by the end of May. The changes in the number and diversity of indigenous microorganisms are being monitored in these experiments and preliminary results using the community fingerprint technique will be completed before the conference. It is expected that this project will be completed in the next twelve to eighteen months.

# MOLECULAR EVOLUTION OF THE RDL GENE IN INSECTS

**Susan Glueck**
Department of Entomology
Cornell University

**Goal:** To synthesize available data on the insecticide resistance gene *Rdl* to better understand the overall structure and function of the gene and its mode of evolution, especially in Dipteran insects.

**Rationale:** The *Rdl* gene, which imparts resistance to the insecticide dieldrin in *Drosophila melanogaster,* was the first invertebrate GABA (gamma-aminobutyric acid) receptor gene to be cloned. GABA is the principal inhibitory neurotransmitter in both the vertebrate brain and central nervous system of insects. The gene was also shown to play a significant role in resistance to the insecticide cyclodiene; a single Alanine--to-Serine mutation in a particular region of *Drosophila* Rdl was sufficient to increase resistance to an entire class of common pesticides. Due to their overuse, many such chemical control agents have been rendered ineffective by unintentional artificial selection for resistance phenotypes in crop pests. When Rdl was sequenced in a number of other agriculturally important crop pests, all of them were shown to have the same resistance-conferring mutation as the one demonstrated in *Drosophila.*

Much of recent research on *Rdl* has focused on a single region of the gene associated with insecticide resistance, without examining the role the rest of the gene plays in coding for an altered form of the protein. This research focuses on the evolution of *Rdl* gene structure in a number of related insects, several of which are of agricultural importance. These include the house fly *Musca domestica,* the Australian sheep blowfly *Lucilia cuprina,* the Mediterranean fruit fly *Ceratitis capitata,* and the Colorado potato beetle *Leptinotarsa decemlineata.*

A comparison of the overall structure of *Rdl* will provide insight into possible functionally important domains that have not yet been considered and may further elucidate the mechanism by which mutations in the gene confer resistance to a wide array of cyclodiene insecticides. In addition, by comparing orthologous sequences in related organisms, this research will contribute to an important area of study in molecular evolution.

**Approach:** The homologues of *Rdl* in four insect species are being cloned and sequenced from both cDNA and genomic DNA. The resulting sequences are compared using computer sequence alignment programs to identify regions of similarity and variability. The phylogenetic importance of certain sequences is being assessed to understand how evolution affects *Rdl*.

**Status:** Over two-thirds of the research for this project have been completed; the remainder is scheduled for completion in the next six months. Data analysis is currently underway.

# EXTINCTION DYNAMICS OF ENDANGERED WETLAND MOTH SPECIES

**Paul Z. Goldstein**
Department of Ecology and Evolutionary Biology
University of Connecticut

**Goal:** The goal of this project is threefold: First, to examine the evolutionary ecology of extinction within a large genus of locally rare and threatened species of moth associated with a variety of equally threatened wetland and other communities; second, to examine the historical extent of threatened maritime and cane-brake wetland communities and most broadly, to investigate the potential for molecular phylogenetic information to inform priority-setting in large scale community- and ecosystem-level conservation.

**Rationale:** The biology of endangerment has not been widely explored from both evolutionary and ecological perspectives. Ecological communities are often treated, both legislatively and practically, as renewable resources that can be effectively restored. Applications of ecosystem management and ecological restoration have relied primarily on current and recent historical patterns of species distributions rather than microendemic pattern (*i.e.,* the unique evolutionary histories of native species at scales where conservation can feasibly be practiced) to inform conservation priorities. This project focuses on North America's largest endemic moth genus, *Papaipema* (Lepidoptera: Noctuidae), a group that contains more threatened and endangered species than any other moth genus in the United States. As many as three species are believed to be extinct, and several are endemic to particular states. Most of these moths feed as caterpillars exclusively on a single plant species, many of which occur only in various types of wetlands. Because of their extreme sensitivity to environmental change and their reliance on specific habitat types, host plant species, and hydrologic regimes, a reconstruction of the relationships among *Papaipema* species combined with distributional and ecological information can help provide a conservative, qualitative estimate of the historical distribution of rare habitats and may help inform priority and feasibility decisions regarding the restoration of threatened habitats.

**Approach:** The following questions are to be addressed: (1) Can some of these threatened communities be identified as biological refugia? (2) Are the most endangered moth species in this genus closely related? and (3) If so, can their threatened status be attributed to a characteristic (such as an association with a particular food plant or hydrologic regime) inherited from a common ancestor, or is it best explained in strictly ecological terms such as habitat destruction? Molecular markers, specifically mitochondrial and nuclear DNA sequence data, are used to reconstruct the evolutionary history or phylogeny (the relationships among species) of *Papaipema.* Following reconstruction, this history will be examined in the context of these species' apparent vulnerability to extinction and their associations with various threatened plant communities. This involves inspecting the phylogenetic tree and mapping the associations with particular plants and community types onto it. From this information, the number of times a given association has evolved, the degree to which it can be correlated historically with a given species' decline, and, if possible, the ancestral distributions of threatened community types in North America will be inferred. The preliminary phylogeny obtained from data generated thus far suggests multiple evolutionary origins of wetland association, multiple evolutionary origins of toxic-plant feeding, one or two origins of fern-feeding, and an origin of the genus in the southeastern United States, possibly in formerly extensive, now endangered cane-brake habitats

**Status:** Forty-two described species of *Papaipma* as well as five undescribed species have been collected or reared, and DNA has been extracted and sequenced from all of these. Sequencing of the mitochondrial cytochrome oxidase II (COII) gene is expected to be completed this spring, and it is hoped that sequencing of two nuclear genes will be complete by 1998, when the analyses will be completed and written up.

# THE EFFECT OF IRRADIANCE AND ZOOPLANKTON ON THE STABLE CARBON ISOTOPIC ($\delta^{13}C$) COMPOSITION ON SCLERACTINIAN CORALS

**Andrea G. Grottoli-Everett**
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University of Houston

**Goal:** To evaluate the effect of solar irradilance and plankton on $\delta^{13}C$ levels in coral skeletons in order to reconstruct paleoclimate (climate of past ages) changes related to cloud cover and the extent of upwelling events in the eastern Pacific Ocean.

**Rationale:** The ability to predict future changes in climate depends on the reliability of Global Climate Models (GCMs). Currently their reliability is limited because they are based on observations that do not date back further than 50-75 years and because the observations were made from primarily terrestrial locations.The lack of data from tropical ocean latitudes is significant because oceans play a major role in controlling climate by serving as a sink and source for heat, carbon dioxide, and other gases. Because instrumental data are largely unavailable in tropical regions, researchers must depend upon climate records chronicled in coral skeletal isotopes. With the $\delta^{13}C$-derived information on light and upwelling developed through this research coupled with $\delta^{18}O$-derived temperature through time, tropical paleoclimate can be accurately described and its natural variability incorporated into GCMs.

**Approach:** The proposed experiments were designed to test the following hypotheses: (1) as irradiance increases, skeletal $\delta^{13}C$ increases; and (2) as plankton levels increase, skeletal $\delta^{13}C$ decreases. A controlled tank experiment was conducted in Hawaii to grow coral fragments of *Porites compressa* and *P. lobata* under specific light and zooplankon regimes for four months. The resulting skeletal material will be extracted, and the $\delta^{13}C$ levels will be analyzed. A multiple linear regression of the data will be used to assess the association between skeletal $\delta^{13}C$, light, and plankton. The resulting predictive model will facilitate reconstruction of past light and plankton levels based on patterns of the skeletal $\delta^{13}C$ signature. The model was validated by conducting a field experiment (similar to the tank experiment); its range of applicability was tested by conducting a similar field experiment in Florida. The model will be used to reconstruct cloud cover and upwelling events from published coral isotope records. The complete reconstruction of tropical paleoclimates and its incorporation into GCMs are essential to improving the reliability of GCMs and their powers of global climate prediction.

**Status:** The tank experiment and corresponding field experiment at the Hawaii Institute of Marine Biology are complete. The remainder of 1997 will be spent extracting and analyzing the skeletal material from the Hawaiian corals (tank and field experiments) and Florida corals (field experiment).

# SPATIAL AND TEMPORAL NURSERY DELINEATION FOR *CARCHARHINUS PLUMBEUS* IN CHESAPEAKE BAY, VIRGINIA, U.S.A.

**R. Dean Grubbs**
Department of Fisheries Science
Virginia Institute of Marine Science
College of William & Mary

**Goal:** To delineate nursery areas used by juvenile *Carcharhinus plumbeus* in Chesapeake Bay through analysis of temporal and spatial habitat utilization patterns in relation to environmental variables such as water temperature, dissolved oxygen, and salinity.

**Rationale:** Chesapeake Bay is the largest nursery in the western Atlantic for *C. plumbeus*, a large sandbar shark constituting over 65% of the sharks landed by the commercial shark fishery along the Atlantic coast. Life history traits such as slow growth, late maturity, and low fecundity (abundance and rapidity of offspring production) render the sharks susceptible to over-exploitation by directed fisheries. As stocks of large coastal species, including *C. plumbeus*, have been severely depleted in the western Atlantic, information concerning stock and recruitment relationships and critical habitats is paramount to proper management. The spatial and temporal delineation of nursery grounds for these species has been designated as a critical data need by the National Marine Fisheries Service. This study delineates the largest nursery for the most abundant and commercially important species in the management unit. The data are essential for future management discussions and the development of regulations protecting vital nursery habitats.

**Approach:** Samples were collected using longlining gear. Each longline set covered a distance of 2-3.5 km and contained 80-140 baited gangions with hooks. Soak time for each set was 3-4 hours. Abundance for each set was estimated by totaling the number of *C. plumbeus* caught per 100 hooks and multiplying the result by 100.

Spatial delineation of the nursery was determined from 70 sets conducted at 37 stations throughout Chesapeake Bay from 1993 to 1996. Temperature, dissolved oxygen, and salinity of the water were measured from surface to bottom at each station. The relative abundance of *C. plumbeus* that was determined was used to identify primary and secondary nursery regions within the bay. The observed distribution of *C. plumbeus* was explained by correlating the abundance data to the measured parameters.

Temporal delineation of the nursery was accomplished by sampling two standard stations within the primary nursery from May through October in 1995 and 1996. Data for 1990 to 1994 for the same stations from the historical longline data were combined with this data to better delineate the temporal usage of the area. Sea surface temperature data were used with the observed abundance data to explain the observed temporal abundance trends within the nursery.

**Status:** Abundance sampling and further delineation of the *C. plumbeus* nursery will continue for two years. The healthy juvenile *C. plumbeus* are being tagged with a juvenile shark dart tag. The tag and recapture data will be used to estimate the nursery's population, quantify stock and recruitment relationships, and examine long-term movement patterns of the juvenile sharks. This summer, some sharks will be fitted with ultrasonic depth-sensored transmitters and will be tracked to determine habitat utilization patterns within the nursery.

# ASSEMBLAGE STRUCTURE AND SPECIES RICHNESS OF 'O'HIA *(METROSIDEROS POLYMORPHA)* CANOPY ARTHROPODS: PRELIMINARY RESULTS AND FUTURE DIRECTIONS

**Daniel S. Gruner**
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University of Hawaii at Manoa

**Goal:** To determine the relative influence of evolutionary and local processes to the structure of arthropod assemblages in Hawaii.

**Rationale:** Until recently, the paradigm of community ecology has been that local processes determine the degree of diversity of natural communities. According to this view, biotic interactions such as competition and predation and abiotic processes such as local disturbance dictate limits to species diversity in a deterministic fashion. Increasingly, it is apparent that processes operating at the regional level, including biogeography and speciation, may enrich the local community as well. To predict the effects associated with global warming and habitat fragmentation, the processes generating and maintaining diversity at both regional and local scales must be understood.

**Approach:** The Hawaiian Islands are an excellent model for community and ecosystem studies. The islands represent a well-defined time series from the northernmost and oldest island of Kauai to the southernmost and most volcanically active island of Hawaii. The fauna and flora of these islands are depauperate (*i.e.*, they fall short of natural size) compared to their continental tropical counterparts; thus, it is possible to study communities in their entirety without arbitrarily ignoring taxa. This investigation focuses on the arboreal arthropod community of the tree *Metrosideros polymorpha,* which exists on all the major islands in monodominant stands. Comparisons of community structure across historical (among-island) and ecological (within-island) gradients will be made.

To examine the regional component of diversity, quantified, standardized canopy fogging will be conducted to obtain arthropod samples. Guild structure and species diversity will be compared among sites and islands. The results of this study will be used to assist the Hawaii Biological Survey in recording and describing the complete fauna of Hawaii.

A combination of sampling and experimentation will be employed to examine the relative importance of predation and resources forces in shaping local herbivorous insect communities. Quantitative branch clipping samples will be collected, and insect abundance and diversity will be correlated to leaf attributes that may vary among treatments. Simultaneous exclosure experiments will be conducted to determine the effects of predators on the insect assemblage.

**Status:** A pilot fogging study was conducted in Volcanoes National Park in October 1996. Fourteen trees were selected to represent ranges of precipitation, age of volcanic substrate, and elevation, factors which vary independently and are expected to affect arthropod community composition. The results of this exploratory study will be used to plan future research.

# ASSESSMENT OF CRITICAL SOCIOCULTURAL AND ECOLOGICAL VARIABLES FOR ADAPTIVE WATERSHED MANAGEMENT

**Geoffrey Habron**
Oregon Cooperative Fishery Research Unit, Department of Fisheries and Wildlife
Oregon State University

**Goal:** To determine current landowner participation in and spatial distribution of watershed conservation projects, focusing on characteristics of participating and non-participating landowners and sociocultural, ecological, and geographic constraints to adaptive watershed management.

**Rationale:** Aquatic resources throughout Oregon have declined to historically low levels in recent years. Without landowner input and support, watershed management may not reflect local needs and may fail to stop the decline of aquatic resources. By soliciting landowners' perspectives, more effective, participatory approaches to adaptive watershed management will be developed.

**Approach:** Agricultural land users in three watersheds of the Umpqua River drainage basin will be studied. The names of landowners who have implemented streamside conservation projects will be obtained from various agencies, and names of both participants and non-participants will be obtained from county tax assessment files. The names will be linked to actual geographic land parcels using county tax assessor digital data and will be combined with other digital data such as stream network, soils, topography, and stream survey data.

A rapid reconnaissance survey approach will be used to select 10 landowners to survey in each of the three watersheds. The landowners will be asked what they consider to be important riparian issues and what current, past, and future riparian conservation strategies they are considering. Based on the results of this survey, a more comprehensive survey of current landowner conservation practices will be conducted in each watershed. Landowners will be asked if they have adopted or will adopt various conservation practices and why. They will also be asked their attitudes toward such practices and their suggestions for future conservation efforts.

The survey will consist of a large mail survey and a smaller personal interview survey. Every survey will be individually geocoded to enable spatial linkage of results. In this manner, social data will be linked to geographic locations within the watershed to determine the spatial distribution of social attributes. The data will be combined in a geographic information system (GIS) to identify factors influencing landowner participation in and satisfaction with conservation projects. Using the GIS, the relationships among the social, physical, institutional and ecological variables will be studied to determine how the attitudes of landowners relate to adoption of watershed conservation practices.

**Status:** Clearance from the Human Subjects Review Board to conduct the research has been obtained, and a review of the questionnaire is complete. Interviews of landowners will begin in April. The physical components of the GIS will be subsequently compiled, and the mail survey will be conducted.

# BIOAVAILABILITY OF HEAVY METALS IN SOILS: A SEQUENTIAL EXTRACTION PROTOCOL USING ARTIFICIAL HUMAN FLUIDS

**Stephanie Hamel**
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Environmental Sciences Department, Rutgers, The State University of New Jersey
and UMDNJ-Robert Wood Johnson Medical School

**Goal:** To develop a laboratory protocol for estimating the bioavailability of heavy metals from soils.

**Rationale:** Heavy metals in soil pose a potential human health risk if ingested by small children. A sequential extraction protocol was developed to replace traditional acid-extraction procedures and better estimate the bioavailability of metals. With better information on bioavailability, potential health risks from any soil can be ascertained, and prioritization of hazardous wastes sites for remediation can be improved.

**Approach:** Human gastrointestinal physiology will be replicated as accurately as possible to estimate human exposure to metals in contaminated soils. The technique to estimate exposure will utilize artificial saliva, gastric juice, and intestinal fluid for determination of the fraction of metals in a soil that is soluble in the gastrointestinal tract. Arsenic, lead, chromium, nickel, and cadmium concentrations will be analyzed using inductively coupled plasma-mass spectrometry. Soil materials, including a National Institutes of Standards and Technology soil, a chromium-contaminated soil, and a lead-contaminated soil were investigated.

The protocol involves a soil recapture technique that provides information for a complete mass-balance. The model will be validated using the EPA Lead Model and comparing it to human lead study data.

**Status:** The initial investigation of the gastrointestinal tract parameters is complete. Liquid-to-soil ratio comparisons and time studies have been addressed, and a paper has been submitted for publication. The mass-balance studies are underway but need to be repeated on other soil materials.

# FEMINIZATION IN COMMON TERNS *(STERNA HIRUNDO)*: RELATIONSHIP TO PERSISTENT ORGANIC CONTAMINANTS

**Connie Hart**
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Woods Hole Oceanographic Institution/
Massachusetts Institute of Technology

**Goal:** To examine the link between endocrine disruption (the feminization of males) in common terns and exposure to contaminants in the environment.

**Rationale:** There is currently much concern over the possible effects of endocrine-disrupting contaminants in both wildlife and humans but little data from real examples exist to determine the potential risks. By studying endocrine disruption observed in common terns, the dangers posed by endocrine-disrupting contaminants can be better understood. As a result, the risks to both wildlife and humans can be better controlled and prevented.

**Approach:** The presence of gonadal abnormalities in common terns from Bird Island in Buzzards Bay, Massachusetts and a reference site on outer Cape Cod will be investigated, and the relationship of abnormalities to environmental contaminants will be assessed. In June and July of 1994, common tern embryos were collected from Bird Island and the reference site. In 1995, pre-fledgling common terns were sampled from Bird Island. Testes of male embryos and pre-fledglings were processed into histological (tissue) slides and examined for abnormalities. Yolk sacs from embryos and eggs from the same nests as pre-fledglings were collected as a measure of chemical exposure of the individual birds. Extracts were prepared from yolk sacs and eggs and were chemically analyzed for a suite of polychlorinated biphenyls (PCBs) and pesticides. The extracts were used to determine dioxin equivalents through a chick embryo hepatocyte (liver cell) bioassay based on EROD (ethoxy-resorufin-o-deethylase) activity. Extracts are also being analyzed for estrogenic activity using an estrogen receptor binding assay. EROD activity was measured in livers of embryos as a biomarker for exposure to PCBs and dioxin-like compounds in the bird. Other tissues and endocrine organs were preserved from common tern embryos for examination. Relationships between gonadal feminization and the different contaminants and groups of contaminants are being analyzed.

**Status:** The histological examination of the pre-fledgling gonads and the estrogen receptor binding assays on extracts from embryos and pre-fledglings are ongoing. A collaboration with the Canadian Wildlife Service was begun in 1996 to examine tern populations in Canada in addition to Bird Island.

# CATABOLITE REPRESSION CONTROL IN *PSEUDOMONAS*

**Kathryn L. Hester**
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University of Oklahoma Health Sciences Center

**Goal:** To identify the factors involved in catabolite repression control (the ability to preferentially metabolize one carbon source over another, thereby repressing induction of catabolic pathways) of *Pseudomonas putida* branched chain keto acid dehydrogenase (BCKAD).

**Rationale:** Pseudomonads are soil and water organisms which play key roles in the metabolism of natural and man-made organic materials, and interest in the genus has steadily increased because of its potential to produce useful industrial products and detoxify chemical wastes. To assess the risks involved in the environmental release of recombinant DNA-engineered *Pseudomonas* strains, molecular characterization of the regulation of gene activity will require extensive investigation. Although much is known about catabolite repression control in *Escherichia coli*, little is known about this process in *Pseudomonas,* except that it does not require cyclic-Adenosine Monophosphate (cAMP). *P. putida* BCKAD is subject to complex regulation by carbon sources and should serve as a good model system for studying catabolite repression in pseudomonads.

**Approach:** The only protein known to be involved in catabolite repression control in *Pseudomonas* is the *crc* gene product of *P. aefliginosa,* which has not yet been isolated from *P. putida. Crc*'s involvement in glucose and succinate repression of *P. aeruginosa* BCKAD will need to be established first. There are two possible molecular mechanisms for *crc*'s involvement in carbon regulation of BCKADs: *Crc* could act as a DNA-binding repressor, or it could bind to a transcription factor (TF) required for transcription of the *bkd* operon or of *bkdR,* which encodes the transcriptional activator of the *bkd* operon. *Crc*'s DNA-binding ability will be assessed, and if none is identified, protein-protein interaction studies will be attempted with *crc* and the TF. *Crc*'s ability to interfere with the positive regulatory protein's ability to activate transcription will be assessed by *in vitro* transcription studies.

**Status:** By determining BCKAD activities of wild type *P. aeruginosa* and *crc*-mutant cultures grown in the presence of various substrates, the *crc* protein was shown to be responsible for glucose and succinate repression of BCKAD. ß-galactosidase activities of a P. *putida* strain were measured, and glucose was shown to repress *bkdR* transcription, suggesting that *crc* is involved in repression of *bkdR* transcription. No DNA-binding activity could be identified for *crc*, so the focus of the project now turns to isolating a TF required for *bkdR* transcription. The current approach is to identify the TF using assays of crude extracts grown under inducible and repressing conditions; the TF will then be isolated, and reverse genetics will be used to clone the gene. Once the TF has been cloned and sequenced, it will be used for studying protein-protein interactions and *in vitro* transcription assays as described above. This work should be completed in 1.5-2 years.

# STRATOSPHERIC DESCENT RATES WITHIN THE POLAR VORTEX

**Jeffrey Hicke**
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University of Colorado at Boulder

**Goal:** To calculate stratospheric descent rates at various times and locations in the Southern Hemisphere using several temperature, water vapor, and ozone data sets and a radiative transfer model.

**Rationale:** A possible explanation of observed mid-latitude ozone loss in the stratosphere is that air which has been primed for ozone loss within the polar vortex is mixed out to lower latitudes. The contribution of this explanation to the actual loss depends strongly on the rate of descent within the polar winter vortex. Mid-latitude ozone loss is much less than loss within the "ozone hole" and not as well understood, but is potentially more significant. The increasing solar elevation angle with decreasing latitude enhances the effect of the ozone loss with respect to increased surface UV radiation, and there are more living organisms, including humans, at mid-latitudes.

**Approach:** The descent rate within the polar vortex using several radiative transfer models and newly acquired data sets has been investigated. The primary radiative transfer model used is the NCAR CCM2 general circulation model radiation code. A more accurate but computationally expensive model called MODTRAN was also used for sensitivity tests and comparisons. Three data sets have been used in the cooling rate calculation. The first data set consists of 1994 temperature retrievals from the airborne HIS (High resolution Interferometer Sounder) instrument in the antarctic polar vortex. These profiles extend from the surface to the aircraft altitude in the lower stratosphere. They are valuable as an independent comparison with data sets used in previous studies of the descent rate. Data from numerical weather prediction model analyses make up the second data set. The United Kingdom Meteorological Office (UKMO) analyses are primarily used in this study due to the model's emphasis on the middle atmosphere. The final data set contains balloon soundings at McMurdo Station, Antarctica. Descent rates have been calculated from these data sets, and comparisons to the UKMO analyses have been made. Results of clear sky calculations, as is typical of previous studies, show lower descent rates.

**Status:** Descent rates from the HIS instrument, balloon soundings, and numerical weather prediction models have been analyzed. Descent rates using these new data sets and clear skies are comparable to other studies, and the contribution to mid-latitude ozone loss from these calculations appears to be minimal. However, it has been shown that both tropospheric and stratospheric clouds can greatly influence the descent rate, so availability of cloud data sets to incorporate into computations will be investigated. This research should be complete within two years.

# INTERACTIONS BETWEEN TOBACCO MOSAIC VIRUS AND THE *N* GENE

**Steve Holzberg**
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University of California, Berkeley

**Goal:** To identify the component of tobacco mosaic virus (TMV) which elicits the *N* gene defense response in tobacco, and to characterize the interaction at the molecular level.

**Rationale:** The resistance or susceptibility of a plant to many pathogens is governed by the interaction of a single plant resistance (R) gene with a single avirulence (Avr) gene in the pathogen. There is evidence that R genes are receptors for either the direct or indirect products of Avr gene, and that this interaction initiates the induction of the defense responses necessary to prevent infection. These defense responses appear to be similar regardless of the nature of the pathogen and to be conserved throughout the plant kingdom. This similarity suggests opportunities for the engineering of genetic resistance in plants to produce novel, environmentally sound options for agricultural disease management. An understanding of the molecular events involved in the specificity of pathogen recognition is fundamental to this effort. The recently cloned tobacco *N* gene is sufficient to confer resistance to TMV. Both sequence and mutational analysis of *N* suggest that it functions as a receptor and signal transducer. However, it is unclear which viral components are required for *N* elicitation and whether these interact directly with the *N* protein.

**Approach:** Two genetic approaches are being used to identify the elicitor of the *N* gene. Both approaches involve the expression of individual viral genes within tobacco plant cells and can be used to test any viral gene, or portion thereof, for elicitor activity. The elicitor will be identified as the smallest region of the virus which is capable of triggering cell death in NN plants but which has no effect on nn plants. The first approach, a series of plasmid DNA constructs designed to express different parts of the virus, will be introduced into plant cells by agro-inoculation. With this method, a solution of agrobacterium harboring one of the viral constructs is injected into leaves and allowed to transfer this genetic material to plant cells in the inoculated region. Once inside the plant cell, the construct will be expressed viral proteins and/or RNA. If the viral construct contains the elicitor, its expression will cause cells in the inoculated region of NN plants, but not nn plants, to die. Constructs that do not contain the elicitor will not cause cell death in either NN or nn plants.

Stable transformation, the second approach, will test the same series of viral genes for the ability to elicit N-mediated cell death. Stable transformation involves the permanent integration of the introduced genes into the plant genome, allowing the introduced genes to be inherited as dominant genes. To avoid regeneration problems, nn plants will be transformed with the viral constructs. These transgenic plants will be crossed to NN plants and monitored for death. The Nn progeny that contain the elicitor gene will die after germination, while those expressing non-elicitor constructs will survive. Because this approach requires much more time to carry out, it will be used largely to corroborate the results of the agro-inoculation approach

**Status:** To date, the agroinoculation approach has been used to define a 50 kDa portion of the replicase protein which is necessary and sufficient to elicit N. Further agroinoculation experiments will determine which features of this protein are important for elicitation. Transgenic plants expressing viral constructs tested by agroinoculation are being crossed, and the progeny are now being grown and analyzed. Currently, the analysis of transgenic lines bearing the control constructs is complete. Experiments are now planned to test for direct interactions between N and the 50 kD elicitor.

# THE EFFECTS OF COMPETITION, HERBIVORY, AND SPATIAL PATTERNS ON A DECLINING NATIVE PERENNIAL GRASS

**Martha Hoopes**
Division of Environmental Studies
University of California Davis

**Goal:** To identify the individual and combined effects of high herbivore densities, competition from non-native grasses, and spatial patterns on the population dynamics and persistence of alkali sacaton (*Sporobolus airoides* Torrey), a native perennial bunchgrass.

**Rationale:** In order to effectively conserve declining species, impacting factors need to be identified and understood. Results of this research should offer concrete recommendations for management of this species and ecosystem while contributing to basic ecological knowledge of invasion dynamics and the synergistic effects of competition, herbivory (plant eating), and spatial patterning.

**Approach:** Growth chamber trials have compared germination of *S. airoides* and two non-native species at different temperatures, salinity levels, and water availabilities. Observations of potted adult plants have suggested that seed set is highly dependent on water availability. A potted competition experiment has suggested that interspecific competition may severely impede recruitment of *S. airoides* but that this effect is mitigated under saline conditions and is most important in the first month after germination. To determine the importance of competition and herbivory on reproduction and survival of adult plants, five adults have been mapped in each of 20 blocks through two summers and will continue for a third summer. Competitor removal treatments have been crossed with herbivore exclusion treatments for four plants in each block with the fifth plant as a cage control. For each plant, species composition in the surrounding 1m x 1m square has been noted, and soil samples for each block have been collected to analyze for salt and nutrient content. A recruitment experiment in caged, uncaged, and partially caged areas offers information on the effects of these factors on seeds and seedlings, Emergence, growth, survival, and reproduction of *S. airoides* will be recorded in each block with interspecific and intraspecific competition as well as in the absence of competition. Surveys of herbivory in patches of different densities and sizes are being used to assess the combined effects of herbivory and spatial patterns. Aerial photographs and GIS data are being used to elucidate larger scale spatial effects, and these GIS data and species composition data will be used to examine inverse correlations of sacaton and different non-native plant species. Simulations of a modified Evolutionary Stable Strategy model suggest that both habitat variability and temporal variability can have very large effects on alkali sacaton persistence. More specific individual-based models are currently being investigated.

**Status:** Most field investigations should be finished by summer 1997. Certain GIS data will be verified in the fall and winter of 1997-1998. If the plants in the recruitment experiment do not flower in 1997, this experiment will be followed through August of 1998. Model simulations, data analysis, and writing should be finished by June of 1999.

# PROPERTY VALUES, CAPITALIZATION, AND THE TAKINGS DEBATE

**Shi-Ling Hsu**
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University of California Davis

**Goal:** To determine whether changes in "regulatory takings" law (the law governing whether a regulatory body must compensate private landowners for regulations that burden or limit the use of their land) have an effect on property values or the intensity of capitalization on private properties.

**Rationale:** Property rights advocates have argued that some governmental regulations are so burdensome to private landowners that they should be compensated for a claimed resulting diminution in value of their property. This position finds intellectual support from those who argue that regulating bodies tend to discount private losses, resulting in an inefficient **under**-investment in property. On the other end of the ideological spectrum, those opposed to compensating landowners argue that such compensation will cause landowners to ignore the social costs of their private decisions, typically resulting in an inefficient **over**-investment in private property. Whether regulatory takings law affects property values is thus a critical threshold question because, if it does not, then no economic efficiency rationale exists for compensating landowners more generously than at present. If takings law has an effect upon property values, then it is also important to determine whether it positively or negatively impacts the intensity of capitalization. The answer to this question could compel a change in the frequency in which private landowners are compensated for use-limiting regulations.

**Approach:** The U.S. Supreme Court has often surprised legal scholars with its decisions on regulatory takings. For example, property rights advocates were pleasantly surprised when the Supreme Court decided *Nollan v. California* and *Dolan v. City of Tigard*, as they represented fairly significant shifts in takings law towards greater frequency of compensation. They were disappointed with *Lucas v. South Carolina Coastal Council*, however, as they expected an Antonin Scalia-led majority to issue a broader ruling than it did. This research will examine coastal property values before and after such important decisions. It should be possible to separate time trends and other market factors from a structural change in land values due to prevailing takings law. In addition to coastal properties, land values of other ecologically sensitive areas which are likely to be subject to regulation will be examined. Agricultural land values will be examined to test whether takings law has an effect upon capitalization intensity, given the sensitivity of agricultural capitalization to the institutional rules which affect farming practices and market conditions.

**Status:** Data are being collected and additional kinds of data that might be available is being ascertained.

# SPATIAL AND TEMPORAL QUANTIFICATION OF BUSH ENCROACHMENT IN OVERGRAZED SOUTH AFRICAN RANGELANDS

**Andrew T. Hudak**
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Cooperative Institute for Research in Environmental Sciences
University of Colorado at Boulder

**Goal:** To quantify the effect of chronic overgrazing on carbon sequestration in semi-arid savannas and to recommend remediatory measures.

**Rationale:** Overgrazing has altered natural fire and herbivore disturbance regimes in formerly grassland savannas, causing unpalatable woody plant densities to increase at the expense of valuable grass forage. This "bush encroachment" phenomenon is perceived as a form of range degradation because it lowers grazing potential and, hence, economic value. Bush encroachment is evident on all continents where cattle grazing occurs but is particularly pronounced in the former Bophuthatswana black homelands in the present Northwest Province of South Africa. In fact, the need for remediation is so great that land use officials of the provincial government have agreed to collaborate in this project.

**Approach:** An automated textural analysis of historical aerial photography will be used to quantify the spatial and temporal extent of bush encroachment across two study landscapes (each of approximately 100,000 hectares). Soil and litter samples gathered at field sites representing various degrees of bush encroachment will be analyzed for total carbon and stable carbon isotope ratio in order to estimate potentially longer-term changes to the soil carbon pool. Aboveground biomass was estimated at these same field sites. Canopy fractional interceptance of photosynthetically active radiation (FIPAR) characterized along 240 m transects will be related to Landsat satellite-derived vegetation indices (VIs). These FIPAR-VI relationships will be used as a tool to scale between field and remotely sensed data. Carbon pools characterized for four major soil/vegetation community types will then be extrapolated across both landscapes using the satellite data and a geographic information system (GIS). Finally, a Markov-type transitional matrix model, driven by cattle stocking, fire, and rainfall variables, will be constructed to simulate changing tree densities. This model will be linked to the Century ecosystem model to further explore how bush encroachment may alter savanna ecosystem processes.

**Status:** All of the required field data were gathered during the fall of 1996, and half of the satellite data have been acquired. The automated textural analysis of the historical aerial photography in a GIS is nearing completion and will be the main topic for this presentation. Satellite image processing will commence in May 1997, while soil and litter sample processing will begin during the following summer. Dissertation is expected to be complete in May of 1999.

# THE ECOLOGY AND GENETICS OF PLANT RESPONSES TO MULTIPLE HERBIVORES

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University of Chicago
Department of Ecology and Evolutionary Biology

**Goal:** To investigate ecological and genetic factors that influence the impact of grazing on plants, and how plants evolve tolerance to this damage, utilizing a natural plant system.

**Rationale:** Livestock grazing is the most widespread land management practice in the western North America, and many argue that it is the greatest threat to the protection of the natural resources of the west. The environmental impact of grazing can be dramatic, influencing both biotic and abiotic components of ecosystems. For example, many experimental studies have documented loss of biodiversity, decreases in plant population numbers, invasion of exotic weeds, and degraded water quality due to livestock grazing on public lands. Most of these impacts are due to the direct effect of herbivory on native plants. Unfortunately, little is known about what ecological factors influence the impact of livestock grazing on native plants and how these practices might be altered to reduce their damaging effects. This is surprising, since recent ecological studies have documented that many native plants can be extremely resistant to *natural* herbivory by deer and elk. Gaining an understanding of the factors which influence tolerance in a natural system will shed light on more appropriate ways to manage livestock grazing and may also influence the development of breeding programs for crop resistance to pests in agricultural systems.

**Approach:** This research will investigate how patterns of genetic variation within and between grazed and ungrazed environments may influence the evolution of plant traits which confer tolerance to herbivory. The basic approach entails large-scale quantitative genetics experiments estimating numerous genetic parameters for plant characters and plant success. This information will allow me to model short-term response to selection using standard multivariate models of evolution

**Status:** Breeding crosses to produce siblings for use in family structured quantitative genetics experiments have been successfully conducted. These experiments are currently being conducted in a natural populations of scarlet gilia, located on the Front Range of the Rocky Mountains in Colorado. Experiments investigating the role of pollen and nutrient limitation on the ability of plants to compensate for early season grazing have also been conducted.

# MYCORRHIZAL ECOLOGY OF *SPOROBOLUS WRIGHTII*

**Linda Kennedy**
Department of Botany
Arizona State University

**Goal:** To identify populations of arbuscular mycorrhizal (AM) fungi that promote fitness and speed recovery of *Sporobolus wrightii* Munro ex Scribn., giant sacaton, on disturbed sites in riparian ecosystems of the Southwest through investigation of the relationship between *S. wrightii* and AM fungi.

**Rationale:** Giant sacaton, a perennial tallgrass found only in southwestern North America, was once a dominant species on many upper floodplain terraces of riparian ecosystems. Today only relics of sacaton grasslands survive due to conversion of upper terraces to agricultural fields. Recent changes in land management have caused many of these fields to be abandoned, and there is strong support to rehabilitate these sites. However, many attempts to restore abandoned fields to sacaton have been unsuccessful, and natural succession has been slow. Inoculation with AM fungi has been an effective tool for promoting restoration in other disturbed ecosystems, but little information is available on AM relationships in riparian ecosystems. Effectiveness of AM fungi varies with species of plant, and populations of AM fungi from separate geographic locations have different impacts on plant hosts, so it is important to determine the response of giant sacaton to AM fungal populations before inoculation is considered as a tool in restoration projects.

**Approach:** This research addresses three questions: (1) What AM fungal species are associated with giant sacaton in different habitats? (2) Do levels of AM fungal colonization in the roots of *S. wrightii* change throughout an annual period? and (3) Will inoculation with AM fungi promote growth of *S. wrightii* seedlings, and if so, is there a difference in effectiveness between populations of AM fungi from different geographic locations? Soil cores from the base of giant sacaton plants growing on upper and lower floodplain terraces of the riparian ecosystem of the San Pedro River in Arizona were collected from four sites at six dates in 1995. Phenological stage of each plant sampled was assessed and percent relative moisture of each soil sample was determined. Sections of root were cleared and stained, and levels of colonization were assessed. Trap cultures have been established using soil samples from the above sites and from floodplain terraces of other rivers in Arizona. Spores collected from trap cultures and from field soil cores are mounted on slides and stained to identify the species of AM fungi found in association with *S. wrightii.* Effectiveness of AM fungal inoculation will be quantified as the difference in growth between seedlings of *S. wrightii* inoculated with AM fungal populations from upper floodplain habitats, lower floodplain habitats, or without inoculum. Data are analyzed with SAS, tested for normality, and transformed if necessary. If assumptions are met, data are analyzed using a general linear models. If assumptions are not met, non-parametric procedures are used. Repeated measures analysis has been used to test for changes over time.

**Status:** Levels of colonization in sacaton roots collected throughout 1995 have been determined. First and second generation trap cultures have been grown from most sites, and identification of AM fungal species is ongoing. The experiment to determine the effectiveness of AM inoculation was redesigned and will be terminated in fall of 1997. Final analysis of data should be completed in the spring of 1998.

# SELECTIVE SORPTION OF POLYDISPERSE ETHOXYLATED NONIONIC SURFACTANTS TO AQUIFER MATERIALS

**Tohren Kibbey**
Department of Civil and Environmental Engineering
The University of Michigan

**Goal:** To examine the mixed sorption behavior of broadly-distributed ethoxylated nonionic surfactants in the presence of various natural materials and develop a quantitative model that would allow prediction of this behavior, in support of developing more effective selection and application of surfactant mixtures for aquifer remediation, as well as fate and transport modeling of subsurface surfactant mixtures.

**Rationale:** In light of the inefficiency of existing aquifer remediation methods, surfactant-based remediation technologies are being considered. Surfactants are able to increase the apparent aqueous solubility of many common organic contaminants by several orders of magnitude; thus, if surfactants were used for pump-and-treat aquifer remediation, they could potentially significantly reduce the time required to remove a contaminant from the subsurface. To cost-effectively apply surfactants to aquifer remediation, however, it is important to understand how surfactant properties influence surfactant behavior in the presence of a given contaminant and aquifer material. One property of commercial surfactants that is often neglected is the fact that they typically consist of mixtures of surfactant components. As such, this study will examine the sorption of surfactant mixtures to aquifer materials to elucidate the influence of surfactant mixture distribution on mixed sorption behavior.

**Approach:** Several ethoxylated linear alcohol and ethoxylated nonylphenol surfactants were selected for this study, some containing as many as 50 separate surfactant components. A high-performance liquid chromatography (HPLC) method was developed to separate and quantify the individual components in the mixtures and was used to study sorption of the surfactant mixtures to a range of aquifer materials. In addition, selected column experiments were conducted to examine the movement of surfactant mixtures through porous media. Based on the observed sorption information, a numerical thermodynamic model was developed. The model can be used to predict sorption of surfactant mixtures with more than 50 components, based on physically-based, measurable parameters, and can also be used to predict the effects of changing surfactant distribution or aquifer material properties on mixed sorption behavior. The model has been incorporated into a 1D transport code and will be used for comparison with column experiments.

**Status:** Anticipated completion date for this project is summer of 1997. Future work in this area will include incorporation of this model into a 2D transport code for modeling the movement of surfactant mixtures during aquifer remediation operations.

# SEDIMENT-WATER PARTITIONING OF MONOMETHYLMERCURY IN CONSTRUCTED AND NATURAL WETLANDS

**Susan Ann King**
Water Chemistry Program
University of Wisconsin-Madison

**Goal:** To quantify monomethylmercury (MMHg) concentrations and distributions in wetland systems; to determine whether dissolved organic carbon (DOC) levels or chemical characteristics correlate with MMHg levels or partitioning behavior; and to determine whether constructed wetlands exhibit mercury biogeochemistry similar to their natural counterparts.

**Rationale:** Monomethylmercury (MMHg) is the most toxic form of mercury and has bioaccumulates in aquatic food chains. Recent research has shown that some wetlands are net sources of MMHg in watersheds, although they act as net sinks for inorganic mercury (Hg) forms. It is not well understood why MMHg appears to be produced in some wetlands and not others, how the MMHg is partitioned between peat, porewaters, and surface waters, and how the aqueous chemistry influences the distribution. Even less is understood about the cycling of MMHg in constructed wetlands.

**Approach:** Concentrations and partitioning behavior of MMHg in peat, porewater, and surface water in two pairs of constructed and natural wetlands will be compared. Water and soil chemistry of the wetlands is being characterizing to evaluate correlations between MMHg levels and partitioning behavior, with emphasis upon DOC quantity and chemical quality. The first wetland pair is in the Florida Everglades water conservation areas. A prototypal stormwater treatment wetland (Everglades Nutrient Removal project) has been constructed there specifically to remove phosphorus from agricultural runoff before it is discharged into the Everglades. The results from this wetland will be compared to a nutrient-impacted natural wetland in Water Conservation Area 2 of the Everglades.

The second wetland pair is in southwestern Wisconsin. A constructed and a natural sedge-meadow wetland exist together in site that was previously characterized hydrologically and chemically by a United States Geological Survey study. These wetlands were chosen for this study because they share similar major ion chemistry but differ in climate, hydrology, and vegetation. These differences cause DOC quantity (and, potentially, chemical quality), bacterial activity, and hydroperiod contrasts between the wetlands being studied. These contrasts should allow evaluation of factors important to MMHg cycling in natural and constructed wetlands.

**Status:** One year of field work has been completed, with the water, soil, and porewater sampling at the Florida site to be completed this summer. Field work at the Wisconsin site will intensify this year and be concluded in the spring of 1998. Research will be complete by December of 1998.

# THE POLITICAL AND SOCIAL FACTORS IN AGRARIAN CHANGE AND FIRE MANAGEMENT IN THE REMNANT TAPIA WOODLANDS OF HIGHLAND MADAGASCAR

**Christian A. Kull**
Department of Environmental Science, Policy and Management
University of California at Berkeley

**Goal**: To understand the social and political context of agrarian life in the Malagasy highlands with respect to landscape management.

**Rationale**: The highlands of Madagascar are celebrated on the one hand for their intensive agricultural landscapes and demonized on the other (largely by environmentalists) for their "barren" and "sterile" treeless landscapes, a result of the fire, deforestation, and soil degradation which "plagues" the island. The highlands were doubtlessly once forested more than today, and only a few patches of highly- degraded woodlands persist. Some of these tapia *(Uapaca bojeri)* woodlands surprisingly exist in areas of long-term and dense human settlement. The tapia woodlands provide the locals with important products such as indigenous silkworms and fruit and are frequently referred to as sacred or protected forests. Meanwhile, grass fires used to renew pastures as well as for political protest and cultural reasons continue to eat away at the edges of the marginally fire-tolerant woodlands. Little is known about the community organization of the use of fire in maintaining pastures or about the role of rural communities in protecting remnant woodlands from the ubiquitous pasture fires. This research will address important questions about the historical relationship between human settlement and deforestation, the relevance of social and cultural factors in forest and fire management, and the evolution in forest and fire management brought about by historical and current agrarian changes.

**Approach**: This research will be based on a variety of ethnographic and archival techniques, primarily the "political ecology" approach advocated by academics such as Piers Blaikie, Nancy Peluso, and Michael Watts. This approach looks at resource management through a variety of theoretical perspectives, including ecological, political-economic, and meaning-based theories, often emphasizing a historical approach. Research will focus on the diagnostic tools of social relations of production, tenure relations, and indigenous knowledge. Detailed ethnographic work (participant observation, interviews, oral histories, and surveys) as well as archival work (examination of historical records of missionaries in the area) will be necessary.

**Status**: The main research will be performed from January to December of 1998. Five months were spent in Madagascar during the Fall of 1996 studying the language, establishing contacts, and prospecting potential research sites. Examination of relevant documents in several archives will begin this summer .

# THE ANTHROPOLOGICAL STUDY OF SCIENTIFIC KNOWLEDGE: THE DIFFICULTIES AND THE POLITICS OF CLIMATE CHANGE SCIENCE.

**Myanna Lahsen**
Advanced Study Program, The National Center for Atmospheric Research
Department of Anthropology
Rice University

**Goal:** To describe the subfield of anthropology known as the "anthropology of science," given the potential contribution this field has made in an age where science often cannot provide clear and certain answers about the nature of the risks with which we live.

**Rationale:** The subfield of anthropology known as the "anthropology of science" is an area of science referred to as "trans-scientific." The study of human-induced climate change is a specific example of trans-scientific inquiry. Knowing the groups and understanding the processes that shape the production and representation of the threat of human-induced climate change is centrally important when interpreting conflicting expert claims and deciding whether to take remedial action. Uncertainty in the science of climate change often results in different conclusions about the reality and seriousness of the threat of future human-induced climate change. In addition, climate scientists often find themselves in a situation where their research may serve or conflict with various political agendas, including their own, and may be appropriated by groups both internal and external to the scientific community.

**Approach:** This poster will map the key actors and outline the issues involved in the production and representation of scientific research that seeks to understand how humans have influenced climate over time.

**Status:** Current research for this project includes conducting, transcribing, and analyzing interviews. The dissertation for this project is expected to be completed by the middle of 1998.

# VASCULAR ENZYME (SEMICARBAZIDE-SENSITIVE AMINE OXIDASE) MEDIATED METHYLAMINE TOXICITY

**Shannon D. Langford**
Department of Pathology
University of Texas Medical Branch at Galveston

**Goal:** To determine the nature of enzyme-mediated vascular injury resulting from exposure to the environmental toxin methylamine.

**Rationale:** Environmental toxins can cause overt vascular toxicity, and many have been implicated in human atherosclerosis. However, few agents have been extensively examined with regard to vascular biotransformation. More exhaustive study of vascular enzymatic interactions with environmental toxins may have far-reaching implications for human disease and toxic exposure. Understanding mechanisms of vascular enzyme-xenobiotic interactions could facilitate the development of future therapeutic and/or preventative measures. Similarly, demonstration of a mechanistic link between environmental toxins like methylamine and human pathology will strengthen regulatory efforts surrounding control of sources such as tobacco smoke and pesticides.

**Approach:** For *in vitro* studies, vascular smooth muscle cells (VSMC) will be harvested from rats via enzymatic dispersion and grown according to standard culture techniques. Methylamine dosage levels will be determined during pilot studies. Toxic injury to VSMC cultures will be determined using assays to determine cell viability and the extent of sublethal toxic injury. Inhibition of Semicarbazide-Sensitive Amine Oxidase (SSAO) will be accomplished with MDL-72274 (Marion Merrel Dow). This compound specifically inhibits SSAO with virtually no inhibition of other amine oxidases. SSAO enzymatic activity will be determined via radiometric microassay.

For *in vivo* studies, mature male Sprague-Dawley rats weighing between 160 and 225 g will be treated daily via gavage (*i.e.*, through a stomach tube) with water only (control), MDL-72274 (blocking control), methylamine (treatment group), and methylamine plus MDL-72274 (blocking treatment group). Duration of treatment and dosage levels will depend on data gathered from preliminary pilot studies utilizing small groups (1 or 2 animals) of rats gavaged with methylamine at various dosages and times.

Because of the possibility of formation of formaldehyde-glutathione (GSH) adducts, urine, blood, aortic tissue, culture media, and cultured cells will be assayed for GSH content and mercapturic acid excretion products. Determination of GSH and formaldehyde-GSH mercapturic acid concentrations will improve understanding of the overall mass balance of toxicant in each experimental system. Histopathology of tissue samples will follow the scoring protocol introduced by Kumar *et al.*, (1990). Statistical analysis of data gathered in these studies will utilize both Student's paired *t* test and one-way analysis of variance (ANOVA); $p \leq 0.05$ will be considered statistically significant.

**Status:** *In vitro* toxicity testing is 90% complete. *In vivo* studies are expected to be completed soon. Expected project completion date is Summer 1998.

# INDUCTION AND INHIBITION OF P450 EXPRESSION IN PISCINE SPECIES: IMPLICATIONS FOR BIOMARKER DETERMINATION AND TOXICITY

**Steven L. Levine**
Department of Zoology
Miami University

**Goal:** To gain an understanding of the relationship between P4501A mRNA levels and P4501 A catalytic rates in order to determine the utility of P4501A mRNA levels as a biomarker of environmental contamination.

**Rationale:** In recent years, reports of carcinomas in North American feral fish populations have increased dramatically. Follow-up studies have correlated the incidence of carcinomas in these fish with elevated sediment, tissue, or metabolite concentrations of organic pollutants, which are most often polycyclic aromatic hydrocarbons (PAHs). Attempts to correlate concentrations of PAHs to carcinomas have been prevented by rapid metabolism of PAHs in fish. The development and validation of biomarkers in fish has become popular in recent years due to the biomarker's potential for quantifying exposure history, providing a temporal framework for exposure, and determining the threshold biological response responsible for the detrimental consequences. Biomarkers on the molecular level offer the advantage of rapid responses to xenobiotics (chemicals foreign to living organisms) and are subject to less interference. Methods for detecting P4501A expression in fish have been refined and include the development of DNA-probes.

**Approach:** This study aimed to characterize the relationship between: 1) hepatic ( liver) P4501A mRNA levels and catalytic activity between the detritivorous gizzard shad and the carnivorous rainbow trout following waterborne exposure to a model P4501A class inducer; 2) catalytic activity between gills and livers—the former having direct contact with the external environment and the latter having indirect contact with the external environment; and 3) P4501A mRNA levels and catalytic activity following exposure to an inhibitor of P4501A catalytic activity. To conduct a practical assessment of induction and inhibition of P4501A expression, continuous waterborne exposures were performed along with realistic concentrations of the P4501A inducer, BaP. The results of this study add to the understanding of the regulation of hepatic P4501A gene expression in fish following waterborne exposure to xenobiotics by: 1) determining the concentration and time-dependent threshold values for induction of P4501A mRNA in gill and hepatic tissue following exposure to BaP; and 2) determining the concentration- and time-dependent effects of the inhibitor propiconazole on hepatic P4S01A expression following exposure to propiconazole alone, or in a combined exposure to propiconazole and BaP.

**Status:** The project is complete. Future work, however, will be conducted to examine: potentiation in the toxicity of the organophosphate insecticide parathion to fish resulting from a pre-exposure to propiconazole, and *de novo* synthesis rates of P4S0lA mRNA with nuclear run-on assays to confirm that the P4S0lA gene is under transcriptional control in piscine species. These additional projects should be completed by the end of 1997.

# EFFECT OF UPSTREAM PROCESS ON UV DISINFECTION

**Frank Loge**
Department of Civil and Environmental Engineering
University of California at Davis

**Goal:** To understand how the operation of upstream processes at a wastewater treatment plant (WWTP) influences the performance of an ultraviolet (UV) disinfection system to enable modifications to full-scale WWTPs so that UV disinfection can become an economically viable alternative to conventional methods of disinfection (*e.g.*, chlorination/dechlorination).

**Rationale:** UV irradiation is increasingly being used as an alternative to chlorine disinfection, but its cost is heavily dependent on wastewater quality. Several empirical UV disinfection models, which can be used to predict the performance of a UV disinfection system given various input water quality parameters (*e.g.*, suspended solids, unfiltered and filtered transmittance at 253.7 nm, and the influent coliform concentration), have been reported in the literature. If the input parameters to these models are the actual parameters affecting the performance of a UV disinfection facility, then one can determine the effect of upstream processes on a UV disinfection facility. However, the specific functional form of a given empirical model changes when calibrated at different wastewater treatment plants, implying that water quality parameters used in the models are "lumped" parameters that can only provide a rough indication of the more fundamental wastewater characteristics affecting UV disinfection. Because of this, it is possible for two upstream processes to have similar "lumped" wastewater characteristics but radically different levels of UV dose necessary to meet the same effluent bacterial concentration. Therefore, fundamental wastewater characteristics affecting the performance of a UV disinfection system must first be identified and then used to evaluate the impact of upstream processes on UV disinfection performance.

**Approach:** This project will involve four steps:.

(1) Develop the methodology for quantifying the fundamental characteristics that influence the performance of a UV disinfection system. These characteristics include: UV-intensity profile in the liquid medium, which will be determined using a mathematical model developed in this research; the degree of particle-association of targeted organisms, which will be determined using a coliform gene probe developed in this research; the UV intensity profile within particles, which will be determined using a fiber optic microelectrode; the response of individual targeted organisms to a known UV dose, which will be developed with pure cultures of coliform bacteria in the laboratory; and the exposure time of targeted organisms to each intensity level, which will be determined with a mathematical model developed in this research.

(2) Develop a quantitative model that incorporates the fundamental wastewater characteristics.

(3) Test the quantitative model with a wide range of wastewater effluents to demonstrate that the fundamental characteristics in Task 1 affect the performance of a UV disinfection system.

(4) Evaluate various upstream design and operating parameters on downstream UV inactivation.

**Status:** Task 1 is anticipated to be completed by June of 1997. Tasks 2-4 are anticipated to be completed June of 1999.

# DOES COPPER INFLUENCE THE DISTRIBUTION OF MARINE CYANOBACTERIA IN THE SARGASSO SEA?

**Elizabeth Mann**
Joint Program in Biological Oceanography
Woods Hole Oceanographic Institution and MIT

**Goal:** To determine whether copper influences the growth rates and distribution of the marine cyanobacteria *Synechococcus* and *Prochlorococcus* in the Sargasso Sea and whether *Synechococcus,* which is present in surface waters with high copper concentrations, is more copper-resistant than *Prochlorococcus.*

**Rationale:** Marine cyanobacteria are widespread and contribute significantly to primary productivity in oligotrophic areas (those deficient in plant nutrients). *Prochlorococcus* alone can account for 30-50% of the chlorophyll *a* biomass and 25% of the yearly productivity in the Sargasso Sea. By comparing the cyanobacteria distribution in the Sargasso Sea to oligotrophic regions in the Pacific, which have lower copper levels, one can gain insights into how the cyanobacterial community might respond to higher concentrations of copper in more polluted environments.

**Approach:** The influence of copper on the marine cyanobacteria *Synechococcus* and *Prochlorococcus* was investigated in the Sargasso Sea. Copper is an essential trace metal, but it is toxic to cyanobacteria in pM quantities; high copper concentrations in the mixed layer were observed to be correlated with low numbers of *Prochlorococcus.* In the laboratory, copper toxicity was evaluated in cultured strains of cyanobacteria. The *Prochlorococcus* isolates fell into copper-sensitive and copper-tolerant groups. The copper tolerant *Prochlorococcus* isolates seemed to be adapted to growth at the surface of the water column. This is not surprising since copper concentrations are highest in shallow mixed layers. However, the growth rates of both copper-sensitive and copper-tolerant *Prochlorococcus* were repressed at free-copper concentrations significantly below those needed to lower the growth rates of *Synechococcus.*

Field data support the hypothesis that *Synechococcus* are more copper-resistant than *Prochlorococcus.* Copper toxicity in the Sargasso Sea was studied using shipboard incubations. The growth rates and abundance of *Synechococcus* and *Prochlorococcus* in control and copper-spiked bottles were compared. Preliminary data indicate that the net growth rate of *Prochlorococcus* in copper-spiked bottles decreased, while the net growth rate of *Synechococcus* in the same bottles did not change.

**Status:** Preliminary data from both laboratory and field tests indicate that copper toxicity has a significant effect on cyanobacteria. Additional work will include growth rate experiments to determine the effect of other metals, such as Mn, on copper toxicity, and field sampling to test for both copper toxicity and iron limitation of *Prochlorococcus.* This research should be completed in about 2.5 years.

# MECHANISMS OF POLYCYCLIC AROMATIC HYDROCARBON-INDUCED IMMUNOSUPPRESSION

**Koren K. Mann**
Department of Pathology and Laboratory Medicine
Boston University School of Medicine

**Goal**: To dissect the pathways involved in the immunotoxic effects of PAHs (polycyclic aromatic hydrocarbons), focusing on the developing B lymphocyte compartment, both *in vivo* and *in vitro.*

**Rationale**: PAHs are ubiquitous environmental toxins, but the extent of human exposure to these chemicals is difficult to measure because of the high rate of PAH metabolism. By elucidating the mechanisms of immunosuppression, innovative biomarkers with increased sensitivity for PAH exposure may be found. In addition, this research may give insights into maladies of exposed individuals, and, in turn, lead to increased awareness of exposure and methods of prevention.

**Approach**: Initially, *in vivo* (inside the living organism) experiments were conducted to determine the effects of a prototypic PAH, 7,12-dimethylbenzanthracene (DMBA), on the bone marrow in mice. At 18 hours after injection, DMBA caused an increase in cells undergoing programmed cell death (apoptosis) when compared to the control. A distinct lack of marrow cells was evident at 48 hours after treatment with DMBA, leading to the conclusion that DMBA affects the site of B cell development by depleting the bone marrow via the induction of apoptosis.

In order to mimic B cell development *in vitro* (outside the living organism), a culture system was developed by coculturing a cloned preB cell line and a cloned stromal cell line. When treated with DMBA, the preB cell lines underwent apoptosis in a dose- and time-dependent manner that was dependent upon contact with the stromal cell layer, as both preB cells grown in suspension and preB cells separated from stromal cells by transwell membranes are resistant to DMBA-induced apoptosis. Thus, not only are the stromal cells the mediators of this DMBA-induced death signal, but contact or close proximity to the stroma is required.

Once PAHs bind to the aromatic hydrocarbon receptor (AhR), it acts as a transcription factor to induce transcription of genes, including oncogenes (cancer-inducing genes) like *c-myc* and *ras* as well as P4501A enzymes. α-Napthoflavone, an antagonist of the AhR and P4501A enzymes, blocks DMBA-induced apoptosis, suggesting a role for the AhR or P4501A enzymes.

Cytochrome P450 enzymes are phase I enzymes involved in metabolizing PAHs, converting lipophilic (fat-soluble) compounds to hydrophilic (water-soluble) compounds, which can be excreted from the body. These P450 enzymes are upregulated by the very compounds they metabolize and create reactive intermediate compounds that are genotoxic and may cause oxidative stress within the cell. A general P450 inhibitor, 1- aminobenzotriazole (ABT), does not block DMBA-induced apoptosis, suggesting no role for these enzymes in PAH-induced apoptosis, but rather the AhR as a mediator of the death signal.

**Status**: Preliminary experiments show that several oncogenes seem to be modulated in the stromal or preB cell after DMBA treatment. Ectopic expression of either of these oncogenes in preB cells may protect from DMBA-induced apoptosis. Therefore transection studies and further characterization of the death signal from the stroma are planned. Also, characterization of bone marrow cells *in vivo,* which apoptose after PAH exposure, will be conducted.

# TESTING FOR DISPERSAL LIMITATION IN A FRAGMENTED EASTERN DECIDUOUS FOREST LANDSCAPE

**Amy B. McEuen**
School of Natural Resources & Environment
University of Michigan

**Goal:** To assess the degree to which plant distributions in a fragmented landscape are determined by dispersal limitation, and to determine the scale at which any such limitation occurs and assess whether the degree of limitation is related to specific plant traits (growth form, dispersal mode, seed size, and seed persistence).

**Rationale:** Determining the degree to which plants are dispersal-limited at the landscape scale is important for assessing the effects of fragmentation and the degree to which plants can move through fragmented landscapes (*e.g.*, in response to climate change). Information on the scale of dispersal-limitation will help determine whether it is important to protect large regional sources or if more widespread protection at a smaller scale is required. Relating dispersal-limitation to plant traits allows predictions regarding which groups of species are at greatest risk in fragmented landscapes.

**Approach:** The approach combines analysis of distribution patterns and seed rain at the landscape-scale with site specific experimentation (*e.g.*, seed additions). The study site, located in St. Clair County, Michigan, consists of a large (<10,000 ha), diverse putative "source" forest surrounded by smaller woods that vary in distance from each other and from the larger forest. The woods will be surveyed to obtain presence/absence data for all woody plants and woodland herbs. For a subset of woods, seed rain will be collected. Plots will be sampled at each seed trap location to obtain relative dominance and abundance data for all woody species. Seed rain data will be related to both within-site plot data and between-site species distribution and abundance. Within-site transects will be established from old regrowth areas and younger recolonized forest to detect declines in forest herbs; seed rain data will be collected in these transects. At isolated sites where selected species show strong declines, seed addition plots (50 seeds/$m^2$) will be set up and monitored for seed disappearance and germination and seedling establishment.

**Status:** Surveys have been conducted for 26 small woods. Presence/absence data have been analyzed for woody species. Isolation effects were stronger when distance to the nearest woods, rather than distance from the putative source forest, was used as the predictor. This suggests dynamics occur at the inter-woodlot, not source-woodlot, scale. For bird-dispersed shrubs, strength of the isolation effect was positively related to seed size. This is consistent with the dispersal limitation hypothesis, since smaller seeded fruits have longer retention times and thus longer dispersal distances. No relationship was found, however, between seed size and isolation effect for wind-dispersed trees. During spring through fall 1997, herbaceous transects will be sampled, woodlots will be resurveyed for spring ephemerals (time permitting), seed rain studies will be initiated, and seed addition experiments will be started. Seed rain traps will be monitored for a year. Seed addition plots will be monitored through spring 1999 (due to two winter dormancy). The project should be completed by the fall of 1999.

# CHICAGO'S LAW AND WETLANDS A CENTURY AGO

**Betsy Mendelsohn**
History Department
University of Chicago

**Goal:** To examine how a categorical and formalist system of law and administration deals with a public good resource by examining how water administration and law were developed during urbanization.

**Rationale:** Politicians opposed to government land takings may use spurious historical claims to argue that the government has never provided environmental goods to the public and that regulation is new and therefore suspect. Although few academics use legal history to refute this argument, this project provides strong evidence that administrative government has the expertise to deal with complex public problems, especially regarding the dynamic natural environment. This project aims to make it more difficult for advocates to simplify history for use in modern political struggles.

**Approach:** Both public and case law are the primary resources for this project. Public law includes statutes and ordinances, as well as administrative rules developed by the Interior and War Departments. Case law is derived from both state and federal courts, which often review the competence of the administrative rules in particular private conflicts. The case files of private conflicts reveal not just judicial opinions, but also the motivations and strategies of litigants and their expectations for justice at law.

The project focuses on two small areas in Chicago—the Southeast side and the mouth of the Chicago River—and will create complex and site-specific histories of these areas, using maps and images as well as text. This project will include a history of individuals dwelling in these areas and will use information from local public bodies, correspondence, and specialty journals that describe the ways people used marshland as an investment and as a place to dwell. A patchwork of marsh and industry persists, defined and defended by law, and challenged by different interest groups who desire divergent destinies for the last vestiges of Chicago's original marsh.

**Status:** To date, several stories that trace law in the executive and judicial branches have been researched and an environmental policy regarding water is being created. Research for this project should be completed in December and writing completed in June.

# MARBLED MURRELET USE OF LANDSCAPES IN SOUTHERN OREGON AND CALIFORNIA

**Carolyn B. Meyer**
Department of Botany
University of Wyoming

**Goal:** To develop a predictive landscape-level habitat model for the marbled murrelet in a region extending from southwestern Oregon to central California in order to determine seascape and landscape variables affecting forest occupancy and identify relationships between offshore murrelet densities and landscape variables.

**Rationale:** The marbled murrelet is a seabird in danger of extinction because it depends on old-growth forests for nesting. It is unknown how fragmentation of old-growth forest habitat and the configuration and proximity of terrestrial and marine features affects the abundance and distribution of this bird.

**Approach:** Murrelet survey data, vegetation databases, and marine data will be analyzed using the ARC/INFO Geographic Information System (GIS) software program; circular plots of varying radii (0.2, 0.4, 0.8 and 1.6 km) will be centered on survey stations; landscape fragmentation indices on the vegetation polygons within the circles will be calculated using FRAGSTATS software; survey station elevation, slope, and aspect will be estimated; and station distance to the nearest habitat feature, including saltwater, island, large bay, dense rocky area, major kelp bed, shore type (rock, sand, mud, gravel), freshwater body, road, edge, river mouth, undersea topographic feature, and cold upwelling areas, will be calculated. In addition, landscape variables between occupied and unoccupied nesting sites will be compared using univariate analysis and logistic regression , and the relationship between standardized bird detection levels and habitat variables will be examined using multiple regression.

Multiple regression will be used to relate murrelet offshore densities to marine habitat features and to landscape fragmentation indices calculated within the corresponding inland landscape. The marine variables include mean temperature, salinity, nutrient concentrations, chlorophyll concentrations, upwelling index, and number of bays, river mouths, promontories (a high point of land projecting into a body of water), and kelp beds.

**Status:** An analysis of fragmentation effects in 203-hectare circular areas around 36 murrelet-occupied forest sites and 36 unoccupied forest sites in southwestern Oregon has been completed. Results indicate that sites occupied by marbled murrelets had more interior habitat within old-growth patches and lower patch diversity than unoccupied sites. Total edge and patch shape were not important. These results suggest that increased access of predators to nest sites may be the cause of the lower occupancy of the fragmented habitats in this area. This study should be completed by December 31, 1998.

# THE RELATIONSHIP AMONG HUMAN POPULATION, ANTHROPOGENIC ACTIVITY AND THE LOSS OF BIOLOGICAL DIVERSITY: A MAYA BIOSPHERE RESERVE CASE STUDY

**Frederick A.B. Meyerson**
School of Forestry and Environmental Studies
Yale University

**Goal:** To examine the effects of anthropogenic activity on biodiversity in the Maya Biosphere Reserve in the Department of Peten, Guatemala by developing a method to efficiently measure changes in species richness of selected taxa and determining statistical relationships between the population-related variables and changes in biodiversity.

**Rationale:** Anthropogenic loss of biodiversity is a function of interactions among human population growth and density, deforestation, and other factors. Like many areas in the humid tropics, the Maya Biosphere Reserve in the Department of Peten, Guatemala, and its rich biodiversity are threatened by rapid human population growth and development both outside and inside the reserve core zones. The population of the Peten has increased from around 21,000 in 1960 to 400,000 in 1996 and is currently growing at an estimated 9-10% per year. The rate of deforestation in the buffer zone was 2.5% per year during 1990-1995. Managers of the Maya Biosphere Reserve are faced with two primary challenges: how to measure and monitor the loss of biodiversity in the face of, and in relation to, this rapid human population growth and development and how to manage the reserve and buffer zones in order to minimize that loss. This project will analyze available policy options and make recommendations as to which courses of action have the greatest potential for reducing loss of biodiversity in and around the reserve core zones.

**Approach:** This core objectives of this project are to: (1) examine the key anthropogenic population-related variables in the human-occupied "buffer zone," which affect biodiversity in the adjacent core zones of the Laguna del Tigre and Sierra de Lacandon parks of the Maya Biosphere Reserve in the Department of Peten, Guatemala; (2) develop a monitoring system and/or set of techniques that efficiently measure changes in species richness of selected taxa in the core in relation to the anthropogenic variables in the adjacent buffer zones; (3) determine what statistical relationships exist between the population-related variables and the changes in biodiversity at selected study sites within the Reserve; and (4) analyze available policy options and make recommendations as to which courses of action have the greatest potential for reducing loss of biodiversity in and around the reserve core zones.

A series of separate sites are being established along the boundary between the core and human-occupied "buffer zones" of the reserve to create a gradient of human population density, growth rates, and distribution "treatments." At each of these sites, biodiversity plots will be established along gradients from the edge of the buffer zone into the core zone of the reserve to monitor both temporal and spatial changes of the species richness of selected taxa (*e.g.*, birds, trees). The extent and rate of deforestation and other anthropogenic variables also will be measured. Where appropriate, other techniques may be employed to understand the sociological causes of population growth, deforestation, and other activity that threatens biodiversity. Data collection for the selected taxa and anthropogenic variables will occur during 1997-1998.

**Status:** In 1996 and early 1997, site visits were made within the Maya Biosphere Reserve to examine potential sites, perform preliminary surveys, and establish partnering relationships with Conservation International and the Nature Conservancy. In the summer of 1997, "treatment" sites will be established and pilot studies performed to select the most appropriate sampling methods.

# MEASUREMENT OF $\delta^{13}C$ IN ATMOSPHERIC METHANE

**John B. Miller**
Department of Chemistry
University of Colorado, Boulder

**Goal:** To devise a system to measure $\delta^{13}C$ (the ratio of $^{13}C$ to $^{12}C$) in atmospheric methane in order to reduce uncertainties in the global methane budget.

**Rationale:** Methane is a chemically and radiatively important trace gas that has been accumulating in the atmosphere at the rate of nearly one percent per year over the last 40 years. However, the distribution and magnitudes of sources are poorly understood. Global measurement of $\delta^{13}C$ in atmospheric methane will allow for a more accurate characterization of sources than is currently possible.

**Approach:** An automated system using gas chromatography coupled with isotope-ratiomass-spectrometry has been developed to precisely analyze air. The automation, small sample size, and short analysis time will enable air analysis from 48 globally-distributed sites of the National Oceanic and Atmospheric Administration's Climate Monitoring and Diagnostics Laboratory's (NOAA/CMDL) Cooperative Flask Sampling Network.. The system will chromatographically separate methane from air on a custom-built cryogenic (-190 to -50°C) pre-column packed with Haysep-D. The eluting methane is then cryo-focused (-190°C) on the head of a capillary column (Molecular- Sieve SA) and separated from residual trace species. Next, the methane peak is combusted at 1150°C, dried, and admitted to the mass spectrometer as $CO_2$. From the combination of global methane mixing ratio measurements (from NOAA/CMDL) and spatial patterns of $\delta^{13}C$, a global transport and a chemistry model will be used to constrain global methane sources more accurately.

**Status:** The system described above has been developed and is operational, except with regard to the automation of the cryogenic parts. This is currently under development and should be completed by the beginning of the summer. Measurements are currently being made from only one NOAA/CMDL site. The rest of the sites will be phased in as automation permits. Use of $\delta^{13}C$ data to constrain the methane budget can only take place after a significant time series (at least one year) has been obtained. The system has been adapted to make high precision (0.1 per mil) measurements of $\delta^{13}C$ and $\delta^{18}O$ of $CO_2$ from air samples as small as 75µl, enabling this technique to be used in the analysis of $CO_2$ in soils and icecores.

# EFFECTS OF SOIL RESOURCES AND INTERSPECIFIC COMPETITION ON PATTERNS OF NON-NATIVE GRASS INVASION IN ARID LANDSCAPES

**Mark E. Miller**
Department of Geography
University of Colorado

**Goal:** To determine the extent to which spatiotemporally distinct variations of *B. tectorum* invasion can be attributed to edaphically (related to soil) mediated patterns of soil-resource availability (water and mineral nutrients) or competitive interactions with native species possessing different physiological characteristics.

**Rationale:** One of the most significant ways in which human activities have transformed landscapes around the world is by facilitating the invasion of native ecosystems by nonnative (exotic) species. In arid and semiarid landscapes subjected to increasing land-use pressures worldwide, invasions by pyrophytic exotic grasses have greatly impacted native ecosystems through the alteration of fire and soil-resource regimes. North American examples include the African perennial grass *Pennisetum ciliare* in the Sonoran Desert and the Eurasian annual grass *B. tectorum* in the Great Basin and Colorado Plateau regions. Knowledge of ecological factors influencing invasions of arid-land plant communities by nonnative grasses is needed to guide future management policies and conservation efforts aimed at ameliorating negative impacts of exotic grass invasions.

**Approach:** Effects of soil resources and interspecific (between species) competition on growth of *B. tectorum* in grassland communities of the Colorado Plateau will be investigated in field experiments conducted at Canyonlands National Park, Utah. During January 1997, 17 replicate experimental blocks measuring 25x25 m were established in grasslands dominated by the native bunchgrass *Stipa hymenoides*. In each block, 12 *Stipa*-centered experimental plots 1.2 m in diameter were established and randomly assigned to one of 12 possible treatment combinations of three, multilevel experimental factors. Experimental factors included: 1) species combination (*S. hymenoides* alone and *B. tectorum* alone versus both species together); 2) soil-nutrient availability (fertilizer added versus no fertilizer added); and 3) soil-water availability (water added versus none added). In each experimental plot, all plants except the target *Stipa* were removed by hand. Plots assigned to *Bromus* treatments were seeded with *B. tectorum* seeds at a rate of 2300 per $m^2$.

Preliminary data and theoretical considerations suggest that growth of *B. tectorum* in arid, quartz-dominated sandy soils common to the Colorado Plateau may be limited by low overall $K^+$ (potassium) availability and the tendency for high levels of divalent cations ($Mg^{++}$, magnesium, and $Ca^{++}$, calcium) to inhibit plant uptake of the monovalent cation $K^+$. To test this hypothesis, fertilized plots were treated with KCl fertilizer at a rate of 30 $g/m^2$. Soil samples were collected to measure among-block variations in physicochemical soil characteristics. Ion-exchange resin bags were buried in plots to measure treatment effects on soil-nutrient dynamics. Plots assigned to receive supplemental water will be watered biweekly to attain two times the long-term precipitation average. Plant-growth and water-potential measurements will be collected at regular intervals from mid-March through the end of June to assess treatment effects on *B. tectorum* and *S. hymenoides*. Plant-tissue samples will be collected in late May to assess treatment effects on plant uptake of mineral nutrients.

**Status:** The 1997 field experiment is currently underway; a comparable experiment will be conducted in communities dominated by the native grass *Hilaria jamesii* during the period January-June 1998. A regional-scale descriptive study to complement the community-scale experimental studies is being planned. Data analyses and syntheses of research results are expected to be completed by Spring 1999.

# REGULATION OF METALLOTHIONEIN GENE EXPRESSION IN THE NEMATODE *CAENORHABDITIS ELEGANS*

**Lori H. Moilanen**
Nicholas School of the Environment
Duke University

**Goal**: To identity molecular mechanisms responsible for regulation of metal-inducible metallothionein (MT) gene expression in the soil nematode *Caenorhabditis elegans*.

**Rationale**: Environmental trace metals can alter gene expression in exposed organisms. A central question in the investigation of metal-responsive genes is how cells sense elevated metal concentrations and transmit this information to target genes. Metal-induced changes in expression of genes for detoxification, stress response proteins, DNA repair, and cell cycle control play a central role in the outcome of exposure. Increased understanding of mechanisms mediating expression of metal-responsive genes will improve our ability to assess and forecast risks associated with exposure. Information gained from this research will also help to improve understanding of metal-induced gene regulation in an ecologically relevant soil organism; enhance interpretation of data from conventional *C. elegans* metal bioassays; and facilitate new approaches to biomonitoring.

**Approach**: The overall strategy is to employ a well-characterized soil organism to probe the regulation of a key gene in metal detoxification and homeostasis. The aim of this research is to identify metal regulatory elements (MREs) within the promoters of two *C. elegans* MT genes and isolate and characterize proteins that interact with the candidate MREs. To do this, the following strategy will be employed:

1) Candidate MREs will be identified by site-directed mutagenesis and deletion experiments. The functional significance of each modification will be determined *in vivo* (inside the organism) by creating transgenic nematodes containing a ß-galactosidase reporter gene. Changes in the level or pattern of reporter gene activity in cadmium-exposed nematodes will be used to identity functional promoter elements. Metal-dependent interaction of crude extract proteins with candidate MREs will be evaluated by gel mobility shift assay and DNaseI footprinting.

2) Regulatory proteins that interact with candidate MREs will be isolated from extracts prepared from control and cadmium-exposed nematodes using conventional protein isolation techniques and affinity chromatography. Binding characteristics of the isolated proteins will be characterized by gel mobility shift assay, DNase I footprinting, and Southwestern blotting assays. Each binding protein will be subjected to partial amino acid sequencing. Complementary DNAs (cDNAs) encoding metal-regulatory proteins will be obtained by screening *C. elegans* expression libraries with oligonucleotides based on the partial amino acid sequence.

**Status**: Work described under Part One above is nearing completion. Two candidate MREs have been identified in the *C. elegans* MT gene promoters by site-directed mutagenesis. Gel mobility shift and Dnase I footprinting experiments to evaluate protein interactions with these sequences are in progress. Work described under Part Two will be initiated early this summer. The dissertation for this research is scheduled for completion by May 1998.

# ESTIMATION OF DISPERSIVITY IN SATURATED POROUS MEDIA

**Jennifer L. Mueller**
Department of Mathematics and Statistics
University of Nebraska-Lincoln

**Goal:** To develop numerical methods for estimating the coefficients in the equation

$$C_t - (D(x)C_x)_x + (v(x)c)_x + q(x)c = f(x,t),$$

which models the transport of dissolved chemical contaminants through saturated soil, where $c(x, t)$ is the concentration of the contaminant and to identify an appropriate set of measurements that lead to an estimation algorithm.

**Rationale:** When such a mathematical model is used to predict the movement of contaminants through groundwater, it is necessary to have an accurate estimation of each of the coefficients. The coefficient $D(x)$ quantifies the dispersivity, a spreading phenomena that occurs as a substance is transported through groundwater. It is caused by the random arrangement of the soil pores and by the microscopic collisions that occur between the particles. Recent experimental evidence has suggested that dispersivity is a function of the distance the contaminant has traveled, and various formulas have been proposed. It is believed that the extra step of solving an inverse coefficient problem to estimate the dispersivity will result in more accurate models.

**Approach:** The problem of estimating $q$ in the steady-state equation: $-C_{xx} + q(x)c = f(x)$ was studied first. This study proved that a sequence of measurements of the flux data $c_x(0)$ corresponding to a certain sequence of source terms $f$ uniquely determines $q$. A numerical scheme based on output least squares (OLS) minimization was developed and implemented. The OLS method finds an approximation to the parameter q by minimizing the difference between the data and the approximation to the data obtained by solving the steady-state equation for a choice of $q$. Since this approach leads to highly oscillatory approximations, it was necessary to develop a regularization scheme to stabilize the problem. The numerical results for this case inspired a study of the sensitivity of the data to perturbations in $q$, and a marked lack of sensitivity was found for certain types of perturbations. The OLS technique also can be applied to the problem of determining the dispersivity $D$ in the original equation. To obtain a well-posed problem, we imposed an initial condition and a mixed boundary condition. In this case, the measured data consist of observations of the concentration $c(x, t)$. While the OLS technique uses the data for explicit comparison, there is another technique, known as the equation error method, which uses the data implicitly in the equation. Both techniques will be implemented and compared in terms of efficiency and accuracy.

**Status:** The study of the steady-state problem has been published and will appear in the journal *Inverse Problems*. The existence and uniqueness of a solution to the forward problem of determining $C$ in the general equation has been established, and a numerical scheme for running simulations of $C$ for various dispersivities is currently being tested. While the two methods for estimating the dispersivity have been formulated, they have yet to be implemented numerically. This work is expected to be completed by mid-July.

# THE PHOTOLYSIS OF NITRIC ACID

**Tanya Myers**
Department of Chemistry
University of Chicago

**Goal:** To gain a fundamental understanding of the molecular processes that influence stratospheric ozone depletion.

**Rationale:** Since nitric acid is a temporary reservoir for the catalytically ozone-destroying $NO_x$ and $HO_x$ species in the atmosphere, its photolysis can influence the ozone balance in the stratosphere. Although several experiments have investigated the competing product channels that occur for nitric acid photodissociated in its intense 190 nanometer (nm) absorption band, there is little understanding of how the molecule is traversing the reaction coordinates to products. There is even disagreement over the product channels that occur and their quantum yields. Surface-mediated photochemistry is important in the atmosphere due to the presence of polar stratospheric clouds. Heterogeneous reactions on polar stratospheric clouds play a central role in the occurrence of polar ozone destruction. Since nitric acid readily adsorbs to the surfaces of ice cloud particles, the photolysis of surface-adsorbed $HNO_3$ molecules can be an important process in atmospheric chemistry.

**Approach:** This project was divided into two parts: (1) measurement of the branching ratio between the competing product channels in the photodissociation of nitric acid at 193 nm; and (2) investigation of the photodissociation of nitric acid adsorbed onto ice crystals to determine its optical absorption properties and photocomposition pathways, which can differ from the gas phase species.

In order to determine photodecomposition pathways, a crossed-laser molecular beam apparatus was used to measure the velocity and angular distributions of neutral photofragments under collisionless conditions. The molecular beam, which is generated by expanding a 4% mixture of gaseous nitric acid seeded in helium into a vacuum chamber, intersects at right angles with a pulsed excimer ArF laser. After the beam intersects with the laser, the photofragments travel 44.1 cm to an electron bombardment ionizer where ionization is induced by 200 eV electrons. A quadrupole mass spectrometer is used to mass select the ions, which are then counted with a Daly detector. The signal intensity of the mass-selected fragment with respect to its time of flight (TOF) after intersection with the laser is recorded with a multichannel scaler. Forward convolution fitting of the TOF spectrum determines the distribution of energies released to the fragments upon dissociation. In order to provide the layer of nitric acid adsorbed onto ice, a pulsed molecular beam source must be incorporated into the apparatus. The ice surface will be formed by freezing water, provided by a continuous molecular beam source, onto a cryogenically cooled substrate. The gas fill in the excimer laser also can be changed to provide 248 and 308 nm light.

**Status:** The first part of the project has been completed. The equipment modifications necessary for the second set of experiments are in progress and should be completed by June.

# MODELING LEAD BINDING TO METAL OXIDE AND ORGANIC COMPONENTS OF PARTICULATE MATERIAL IN NATURAL AND ENGINEERED AQUATIC ENVIRONMENTS

**Yarrow M. Nelson**
School of Civil and Environmental Engineering
Cornell University

**Goal:** To provide a mechanistic basis for the development of models to predict the fate, transport, and bioavailability of toxic trace metals in aquatic environments by quantifying the relative contributions of metal oxides and organic materials to trace metal adsorption by suspended particulate material (SPM).

**Rationale:** Many aquatic environments in the United States are polluted with toxic transition metals, which are listed as contaminants at most Superfund sites. The key to predicting the impact of metal contaminants on ecosystems and human health is understanding the processes that control metal bioavailability in the environment. It is well established that metal speciation and adsorption to particulates are the primary controls on metal bioavailability. However, there is still no way to predict the impact of these processes on a given aquatic system using a general model. This research project is expected to provide the basis for generalizing models for trace metal adsorption based on the composition of SPM in a given environment.

**Approach:** A combination of laboratory and field investigations were conducted using Pb (lead) as a model trace metal. Laboratory experiments were conducted to examine Pb adsorption to biological materials and metal oxides that serve as analogs for components of natural SPM, while field experiments were used to characterize SPM in natural and engineered aquatic environments and to measure Pb adsorption to surface coating materials (SCM) collected in the field.

Laboratory analogs for the organic phase of SCM included pure-cultures of diatoms (planktonic unicellular or colonial algae with silica-containing skeletons) and algal cells and a bacterial extracellular polymer; SCM minerals were represented by oxides of iron (Fe), manganese (Mn), and aluminum (Al). Pb adsorption was measured for SCM collected from natural aquatic environments and from wastewater treatment systems. SCM was characterized for total organic material on the basis of chemical oxygen demand, and metal oxides were assayed using inductively coupled plasma spectroscopy. Pb adsorption to components of SCM was used to estimate the relative contributions of organic and metal oxide constituents and the combined estimate was compared to observed Pb adsorption isotherms.

**Status:** Completed research has yielded significant results regarding the relative contributions of SCM components to overall Pb adsorption. Estimated Pb binding to Fe oxides in SCM was greater than that to biological components or to Mn or Al oxides. Predicted Pb binding to the organic phase and Fe, Mn, and Al oxides accounted for 60 to 90% of the total observed Pb binding to the surface coatings. As expected, the surface area of model oxides strongly controlled Pb binding, as demonstrated using several Mn oxides. This approach can now be extended to additional aquatic environments with diverse conditions.

Results thus far have identified several key mechanisms governing trace metal interactions that merit further study under controlled laboratory conditions. For example, the role of biological interactions with Fe oxides in controlling trace metal adsorption by Fe oxides should be elucidated and modeled. Further, trace metal adsorption by biologically oxidized Mn should be investigated because such oxides are expected to exhibit large surface areas. Thus, the role of Mn oxides in controlling trace metal cycling could be greater than indicated by the present work based on crystalline Mn oxides.

# THE STRUCTURE AND EMISSIONS OF PARTIALLY PREMIXED $CH_4$ FLAMES

**Robin J. Osborne**
Department of Mechanical Engineering
Vanderbilt University

**Goal:** To understand the effect of aerodynamics and flame curvature on pollutant formation, identify major species and temperature in basic flame geometries typically found in residential and commercial appliances, and provide an experimental basis for detailed pollutant chemistry models.

**Rationale:** Better understanding of pollutant formation will lead to an improvement in indoor and outdoor air quality, as well as more cost-efficient combustion processes. To achieve this understanding, more experimental research is needed to find the effect of stretch and curvature on the concentrations of major and minor species and temperature within turbulent and laminar (streamlined) flow flames. The results from this research will facilitate the construction of accurate models of the finite-rate chemistry that occurs during combustion. Based on these models, combustion processes can be optimized in several different ways, such as by altering fuel compositions, air/fuel ratios, or temperatures.

**Approach:** In the Laser Diagnostics and Combustion Laboratory at Vanderbilt University, multiple-point measurements of methane ($CH_4$), carbon dioxide ($CO_2$), hydrogen ($H_2$), oxygen ($O_2$), nitrogen ($N_2$), water ($H_2O$), and temperature will be made in laminar, partially premixed methane-air vs. air flames using a laser and a newly designed Raman/Rayleigh spectrometer system. NO and CO concentrations also will be measured using laser-induced fluorescence (LIF) with a tunable, excimer-pumped dye laser. Planar, cylindrical, and co-flow flame geometries will be studied, and the effect of aerodynamic stretch and curvature will be examined. Results from these experiments will be compared to a flamelet library based upon existing detailed chemistry complex transport combustion models.

**Status:** LV Raman scattering measurements have been made in laminar, planar partially premixed methane-air vs. air flames. Major species concentrations and temperature profiles have been extracted from the data and compared to numerical models provided by Yale University. While comparisons show reasonable agreement, the accuracy desired in the experimental results was not obtained because of polycyclic aromatic hydrocarbon (PAH) fluorescence in the Raman spectra. Better background subtraction can be achieved using a visible Raman system with a well-polarized laser. The new visible system is being set up, and the visible Raman/Rayleigh spectrometer system has been designed and is currently being aligned.

# PHOSPHOROUS WATER QUALITY MODEL EVALUATION AND COMPARISON FOR NATURAL AND CONSTRUCTED WETLANDS

**Mary M. Paasch**
Department of Agricultural Engineering
Texas A & M University

**Goal:** To validate and compare phosphorous models for constructed and natural wetlands, characterizing the applicability and limitations of available models.

**Rationale:** The use of natural and constructed wetlands for water quality enhancement and pollution is widespread. However, numerical modelling of long-term phosphorous retention in wetlands has been limited. Several models have been developed to reflect the long-term phosphorous retention for wetlands, including the Vollenweider-type model for wetlands, the mass balance model with first-order areal uptake, and the detailed ecosystem model for constructed wetlands.

**Approach:** Each of the three phosphorous models were evaluated using sets of wetland water quality data from four different wetland sites. Two of the sites were industrial/wastewater treatment wetland systems: the municipal wastewater treatment system in Vermontville, Michigan, and Jackson Bottom Wetlands Preserve in Hillsboro, Oregon. Both of these sites utilized constructed wetlands for treatment of secondary effluent. The remaining two sites utilize wetlands for nonpoint sources: Des Plaines River Wetlands Demonstration Project in Wadsworth, Illinois, and Everglades Nutrient Removal Site in West Palm Beach, Florida. Each model will be run with two years of data from each of the four wetlands sites. One or two cells from each wetland will be used to evaluate each model. The same cells from each geographic location will be used to evaluate each of the water quality models. Validation of the models will be accomplished by comparing the actual data to the corresponding simulated data. The main focus will be on the models' abilities to accurately predict annual phosphorous retention (mass).

**Status:** The project is progressing as originally anticipated. Currently, the models are being run with the above-mentioned data sets. Validation and comparison of the three phosphorous models should be completed by June 1, 1997.

# BEHAVIORAL ECOLOGY OF SYMPATRIC BARRACUDA SPECIES

**Shane H. Paterson**
Department of Zoology
University of Georgia

**Goal:** To examine fragmentation and coalescence of aggregations of barracuda species (family *Sphyraenidae*) in Papua New Guinea, focusing on habitat availability and utilization, and on possible size-related social continuum, heterospecific schooling, and lower site fidelity than is found in specimens from the Western Atlantic.

**Rationale:** A thorough understanding of community function is prerequisite to any ecosystem-based management plan intended to protect a threatened natural resource. Censuses and behavioral studies of top predators should be a priority of any such plan. Piscivores have largely been neglected as the subjects of behavioral research. This study will provide the first behavioral record of at least three barracuda species. Focusing on sympatric congeners (members of one genus occupying the same area) may yield insight into the evolution and maintenance of social in top predators and may apply across a broader range of taxa and habitats.

**Approach:** The primary focus of this study will be on behavioral observations of barracuda and will employ Earthwatch volunteers as field assistants. Behavioral data will be based largely upon use of the "focal animal" and "scan" techniques as well as timed swims (techniques that have been used in previous barracuda research in the Turks and Caicos Islands and Florida Keys). Groups and individuals of barracuda will be observed and the degree of group cohesion and persistence noted. Solitary barracuda will be observed repeatedly to obtain data on site attachment and territoriality. Habitat surveys will allow "focal point" analysis of habitat utilization. Lengthy snorkel observations also will be made at certain sites from early morning to dusk to determine if and when large groups of barracuda fragment and re-form.

**Status:** Research for this project will continue for at least one more summer, at which time a dissertation will be completed.

# THE COCHLEA AS A SPECIALTY COMPARTMENT: TOXICOKINETIC MODELING OF SOLVENT DISTRIBUTION

**Terri A. Pearce**
Department of Occupational and Environmental Health
University of Oklahoma Health Sciences Center

**Goal:** To aid risk assessment by contributing to understanding of solvent ototoxicity.

**Rationale:** Hearing loss can have profound effects on quality of life for individuals. Human epidemiology implicates certain solvents such as toluene, trichloroethylene, and others in ototoxicity (exerting toxic effects on ear neural tissue). Animal studies have further associated ototoxic effects with solvent exposure. Evidence from both forums indicates that solvent exposure in conjunction with noise exposure may lead to more impairment than exposure to either agent alone. The widespread use of solvents underscores the importance of understanding their ototoxic potential. The possible interaction with noise is significant for risk assessment because noise represents a common environmental exposure. This research will lead to a better understanding of hearing loss due to these agents.

**Approach:** Classical toxicological methods do not offer all of the tools necessary to allow for rapid screening of ototoxicants. This means that the information needs of the scientific and regulatory communities along with those of the general public may not be fully met. To meet these needs, toxicologists are expected to adapt classical methods to emerging technologies. One of those emerging technologies is the ability to perform toxicokinetic modeling rapidly and easily. Models can offer initial information which may be verified through classical laboratory methods. This study will seek to adapt one of the existing toxicokinetic modeling software programs to a specialty compartment, specifically the cochlea. It will examine existing models for their ability to predict solvent concentrations found there. Toluene and trichloroethylene will be used in this study, as both have been implicated in ototoxicity.

Current estimates of cochlear concentration, either through models or through laboratory exposures, assume that solvent concentrations found in the brain are representative of concentrations in other neural tissues including the cochlea. While this assumption may be accurate, it has not been validated. This study will seek to address that issue. The models will be compared according to predicted concentrations of solvent in the brain and how those predictions relate to experimental values from the literature. The validity of the model will then be challenged experimentally by injecting animals with radiolabelled solvent. The concentrations found in the cochlea as well as brain, blood, and liver will be determined and the measured values compared to those predicted by the model. This work will substantiate whether it is appropriate to assume that concentrations in the brain mirror those in the cochlea. The final component to the research will be simultaneous solvent and noise exposure to determine whether noise influences the distribution of solvent in the cochlea.

**Status:** Modeling should be finished by the end of Summer 1997. All research including dissertation defense will be concluded by August 1998.

# ENVIRONMENTAL AND TOXICOLOGICAL APPLICATIONS OF CAPILLARY ELECTROPHORESIS

**Phanendrakumar V. Penmetsa**
Department of Toxicology
North Carolina State University

**Goal:** To demonstrate applications for a relatively new analytical technique, Capillary Electrophoresis, in environmental and toxicology studies, examining the diverse separation chemistries available with capillary electrophoresis (CE) for analysis of pesticides and biomarkers of exposure (DNA-adducts).

**Rationale:** As part of his Environmental Technology Initiative (ETI), President Bill Clinton set forth goals for continuous monitoring capabilities to prevent pollution in waste streams. The new technologies to be developed under this initiative must be inexpensive and compatible with a broad range of analytes. They also should generate little waste and minimize analyst exposure to hazardous organic solvents. The aim of this research is to use capillary electrophoresis as a new analytical tool that meets these criteria. The use of narrow bore capillaries (50-μm internal diameter) for separation ensures low waste and minimal use of reagents. CE is applicable to a broad range of analytes and the methods are rapid and inexpensive. Because of its fast separations capability, CE has been suggested as the basis for chemical sensor and micro-miniaturization technology. The diverse separation chemistries available with CE offer specificity and adaptability that are unparalleled by any other single analytical technique.

**Approach:** The novel separation techniques available with CE for analysis of pesticides and biomarkers of exposure were examined. A number of CE methods were developed using different modes of capillary electrophoresis such as capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) for analysis of numerous pesticides in water and soil samples at the part-per-billion (ppb) level. The pesticides investigated were atrazine, simazine, alachlor, metolachlor, primisulfuron, triasulfuron, carbendazim, metalaxyl, propiconazole, and vinclozolin. In addition, highly sensitive methods were developed using capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) for analysis of a few pesticides (2,4-D, dicamba, and chlorimuron ethyl) and Benzo(*a*)pyrene DNA-adducts at the picogram to femtogram levels, respectively. The utility of capillary electrophoresis for obtaining highly rapid chiral separation (separation of left- and right-handed molecular forms) of pesticides using cyclodextrins as chiral reagents was also examined. The chiral pesticides examined were imazaquin, diclofop, propiconazole, bioallethrin, and fenpropathrin.

**Status:** The final stages of research are nearing completion. Research to date has resulted in four publications (two printed and two in press). Two more manuscripts will be prepared for publication. A few experiments still need to be completed. Research and dissertation will be completed by July 1997.

# A FRAMEWORK FOR ASSESSING THE RISKS OF WATERBORNE *CRYPTOSPORIDIUM:* APPLICATION TO ENDEMIC RATES

**Joseph Perz**
Division of Environmental Health Sciences
Columbia School of Public Health

**Goal:** To develop and apply, with available occurrence data, a framework for the comparison of risk assessment-derived estimates of infection and illness rates due to exposure to low levels of infective *Cryptosporidium* in tap water.

**Rationale:** *Cryptosporidium,* an opportunistic pathogen in immunocompromised hosts, is ubiquitous in the environment and is highly resistant to standard water disinfection practices. While waterborne transmission is well established in community outbreaks of cryptosporidiosis, the significance of low levels of occurrence of this parasite in non-outbreak settings remains unclear. Uncertainty regarding the contribution of tap water to endemic cryptosporidiosis relative to other exposures has complicated the formulation of regulations and guidelines for protecting public health.

**Approach:** The application of risk assessment models utilizing available dose response data for *Cryptosporidium* infection can provide estimates of population-based risks from tap water consumption. Comparisons of such risk assessment results with available occurrence data are complicated by the spectrum of clinical responses that is associated with infection with these organisms. It is therefore important to relate infection rates to illness outcomes. Infections occurring in immunologically healthy persons may frequently be asymptomatic or mild and are self-limiting in their course. On the other hand, in immunocompromised hosts (persons with AIDS for example), infection with *Cryptosporidium* tends to be persistent and profoundly debilitating. Therefore, this risk assessment considered persons with AIDS separately. Infection risks were generated using two assumed levels of *Cryptosporidium* occurrence.

A source of data on infection rates is provided by epidemiologic surveillance programs which generally consist of the facilitated reporting of infections from laboratories that perform stool examinations. Typically, testing for *Cryptosporidium* is done only at the request of physicians, with the result that most requests come from clinicians who treat AIDS patients and are aware of the disease. In contrast, cases occurring in the general population, even when associated with medical attention, are unlikely to receive a confirmed diagnosis via stool examination for *Cryptosporidium.* A sequence of events was derived in order to relate infection to illness severity, medical care utilization, diagnostic testing, and case reporting. Quantitative estimates of each of the component events for the AIDS and non-AIDS subgroups were derived and applied to the infection risk estimates. Results are presented in the form of projected case rates for populations with varying prevalence of AIDS.

Uncertainties regarding the actual occurrence of infective *Cryptosporidium* in tap water are substantial. However, the analysis was consistent with the premises that tap water may represent an exposure route for endemic *Cryptosporidium* infection and case counts and rates derived from surveillance may substantially underestimate overall rates of infection and illness. The analysis also indicated that reported levels of cryptosporidiosis—and the preponderance of cases among persons with AIDS—could result from an exposure common to the entire population, irrespective of whether tap water is a source.

**Status:** An oral presentation of this work was made at the International Symposium on Waterborne *Cryptosporidium* in March 1997. Future work will involve molecular approaches to the epidemiology of *Cryptosporidium* infection.

# AN AQUEOUS PHASE CATALYTIC OZONATION PROCESS FOR REMOVAL OF MICROPOLLUTANTS AND OZONE BY-PRODUCTS

**David S. Pines**
Department of Civil and Environmental Engineering
University of Massachusetts, Amherst

**Goal**: To develop an aqueous phase transition metal catalyst that will enhance the ozonation process by accelerating the destruction of micropollutants and ozonation by-products such as biodegradable organic carbon and bromate.

**Rationale**: Many water utilities are considering using ozone instead of free chlorine as a primary disinfectant in order to minimize the formation of chlorinated disinfection by-products. Ozone is a stronger disinfectant than chlorine, and treatment with ozone followed by monochloramine is effective at inactivating *Cryptosporidium*. EPA does not list ozone as a best available technology because of the uncertainty related to ozonation by-products. There is a need to develop technologies that reduce ozonation by-products so that ozone can be used safely as a disinfectant/oxidant in drinking water treatment.

**Approach**: The evaluation of a catalytic ozonation process for drinking water treatment is being performed in three phases. Phase 1 will evaluate a homogeneous catalyst for the oxidation of a model compound, a hydrophilic acid fraction from a filter effluent, and the waste filter effluent itself. A hydroxyl radical probe compound is included in the matrix to determine if the catalytic mechanism includes the generation of hydroxyl radicals. The primary emphasis of this phase is to better understand the catalytic reaction pathway. Phase 2 will emphasize the development of a catalyst for use in drinking water treatment. Various heterogeneous catalysts will be evaluated. If the reaction mechanism also includes the generation of hydroxyl radicals, the water will be spiked with toxic contaminants that are resistant to direct oxidation by ozone. Tests will also be performed to determine if the catalytic process can reduce bromate, since formation of bromate is a major deterrent in using ozone for bromide-containing waters. Phase 3 will be used to study the surface adsorption characteristics of a heterogeneous cat alyst at the molecular level using an in-situ attenuated total reflectance infrared absorption (ATR-IR) method. A structural understanding of the surface complexes will provide information about the reaction pathway and enhance the development of an effective catalyst.

**Status**: The first phase of this research has been completed. Tests showed that cobalt(II) catalyzed the mineralization of oxalic acid, a common by-product of ozonation. At all conditions tested, Co(II) also increased the steady state concentration of hydroxyl radicals. However, Co(II) was not very effective at reducing the dissolved organic carbon concentration of a filter effluent. Additional analysis is required to determine if the catalyst increases the readily biodegradable fraction of organic matter, which would improve the organic removal of a downstream biofilter. Consistent with the oxalic acid test results, Co(II) increased the hydroxyl radical concentration under most test conditions. Therefore, elements of catalytic ozonation may be similar to the advanced oxidation processes.

# CHARACTERIZATION OF A NOVEL GLUTATHIONE S-TRANSFERASE WHICH MAY PROTECT FROM OXIDATIVE STRESS

**John Thomas Piper**
Department of Human Biological Chemistry and Genetics
The University of Texas Medical Branch at Galveston

**Goal:** To investigate and structurally and kinetically characterize the physiological relevance of a novel glutathione S-transferase (GST) isozyme which may play a significant role in the protection from both chemically-induced oxidative stress and the deleterious end products of this process.

**Rationale:** The growing recognition that oxidative stress may play a significant role in the molecular mechanisms of a number of chemical carcinogens underlies the importance of the characterization of endogenous defense systems against oxidative stress. Glutathione S-transferases in general have previously been described as a defense mechanism against oxidative stress. However, the role of this novel group of human GST isozymes, currently designated as hGSTs 5.8, has yet to be delineated. Thus far, the kinetic characteristics of hGST 5.8 suggest that, of the GST isozymes previously described in mammals, this GST isozyme may have evolved specifically as a defense mechanism against oxidative stress. An understanding of the structure/function relationship and factors influencing the expression and activity of this GST isozyme may lead to the development of strategies for the protection from chemically-induced carcinogenesis.

**Approach:** The tissue distribution of hGST 5.8 will be assessed by standard immunodetection techniques. The primary structure of hGST 5.8 will be determined in parallel by both protein chemistry and molecular biology techniques. The liver and aorta forms of hGST 5.8 will be purified to homogeneity and the primary sequence of the purified protein will be determined through a series of digests with subsequent sequencing of the resultant peptide fragments. The cDNA of liver hGST 5.8 will be obtained through screening of a commercially prepared cDNA library using the cDNA of the mouse homolog of hGST 5.8, mGSTA4-4, as a probe. The recombinant hGST 5.8 protein will then be expressed in a prokaryotic (non-nucleated) expression system. Once the kinetic characteristics of the recombinant protein have been established and compared to the native enzyme, extensive kinetic studies using various GST substrates and inhibitors will be performed.

The physiological relevance of hGST 5.8 as a protective mechanism against oxidative stress will be investigated using stable transection of a eukaryotic (nucleated) hGST5.8 expression construct into H-69 cells. Once stable subclonal cell lines have been established, the cells will then be exposed to increasing levels of a variety of compounds known to cause oxidative stress. Cell survival, glutathione levels, and lipid peroxidation products in the form of thiobarbituric acid reactive species will be compared in control and treated cells in order to determine if the over expression of hGST 5.8 can provide increased protection from oxidative stress.

**Status:** Seventy-eight percent of primary structure has been determined by protein chemistry. Screening of cDNA library has not yielded a clone; analysis suggests that the cause is a low abundance of message. Transection studies will be initiated using the mGSTA4-4 cDNA. Expected project completion is Summer 1998.

# CONTROL OF ALGAE IN DRINKING WATERS BY OXIDATION AND COAGULATION

**Jeanine D. Plummer**
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University of Massachusetts at Amherst

**Goal:** To determine optimum control of algae through a combination of oxidation and coagulation.

**Rationale:** Algae in drinking water supplies can cause a number of problems, including unpleasant tastes and odors, clogging of filters, production of DBPs (disinfection by-products), and interference with coagulation processes. The proposed DBP requirements of the SDWA (Safe Drinking Water Act) reauthorization are prompting many water utilities to consider using ozone as an alternative disinfectant to chlorine.

**Approach:** Three algae are used in this research: a green alga *(Scenedesmus quadricauda),* a blue-green alga *(Microcystis)* and a diatom *(Cyclotella).* The algae are brought to steady state growth in the late log phase using a chemostat. Research examines the effect of preoxidation with ozone and chlorine on (1) algal cell properties and extracellular organic matter (EOM); (2) coagulation and separation of algae; and (3) disinfection by-product (DBP) formation for algae.

The experimental design involves four phases: Phase I experiments examine the effect of preozonation on the particulate and organic character of a suspension of algae. Measurements of total and dissolved organic carbon, particle size distribution, UV absorbance, and turbidity are made. In Phase II, the effect of preoxidation on algal cell morphology and on the concentration and composition of EOM are determined. Phase III experiments examine both the kinetics and 7-day formation potential (FP) of trihalomethanes (THMs) and haloacetic acids (HAAs) for algal suspensions with and without preozonation. Samples are dosed with chlorine and incubated under standardized pH and temperature conditions. After incubation, samples are processed and concentrations of DBPs are determined.

Experiments in Phase IV are designed to evaluate oxidant effects on microflocculation. A synthetic water is spiked with algae, pre-treated with ozone, and used in jar tests. Coagulation effectiveness is determined by measurements of organic matter, turbidity, particle counts, and DBP production. The effect of preoxidation on the coagulant dose needed to achieve good coagulation is being studied.

**Status:** Phase I and III experiments are complete for *S. quadricauda.* Prior to any treatment, the THMFP and HAAFP of a suspension of *S. quadricauda* were 62 and 153 µg per 10,000 cells, respectively. With preozonation, the DOC content of the algal suspension increased by up to 400%. This additional DOC, presumably arising from increased liberation of EOM, acted as a precursor for THM production. This was shown in an up to 30% increase in THMFP. Preozonation also affected the speciation of HAAs, with a shift from trichloroacetic acid to dichloroacetic acid. Because preozonation changes the potential for DBP production, effective treatment strategies are necessary to remove algal precursor material before oxidation.

# MOLECULAR CHARACTERIZATION, DETECTION, AND ENUMERATION OF THE HARMFUL ALGAL GENUS *ALEXANDRIUM* (DINOPHYCEAE)

**Nicole Poulton**
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Woods Hole Oceanographic Institution/
Massachusetts Institute of Technology

**Goal:** To explore the applications of "molecular probe" approaches for the detection, enumeration, separation, and genetic characterization of the toxic dinoflagellate genus *Alexandrium* in natural field samples.

**Rationale:** The significant public health, economic, and ecosystem impacts of harmful algal booms (HABs) are the motivations for the proposed research goal. Only in the last decade have the scientific and commercial communities recognized the impact toxic blooms have on all components of the marine food-web, including viability, growth, fecundity, and recruitment. Plankton sampling is now considered a necessary complement to the detection of toxin in affected resources. However, identification and enumeration of harmful species rely on tedious microscope techniques which are time consuming and expensive. Additionally, the regional and global heterogeneity of *Alexandrium* poses serious specificity problems that must be addressed. Other methods using molecular "probes" that provide identification to the genus, species, and strain-specific level have been developed recently. These probes bind to proteins, nucleic acids, or other molecules located inside or on the cell surface of the species of interest. Antisera have provided remarkable specificity in some cases, but cross-reactions are still present for most probe types. In addition, field applications of antibody and nucleic acid probes to HABs have been limited. Therefore, there is an overall need to improve and test the existing methods for characterizing, detecting, and accurately enumerating harmful algal species in coastal marine environments. This work will examine the relative strengths and weaknesses of immunological and nucleic acid-based methods and apply a subset of these in population studies of *Alexandrium*.

**Approach:** This research has three components:

(1) Examine phylogeny and heterogeneity within the genus *Alexandrium*. Using polycolonal and monoclonal antibodies developed against *Alexandrium* spp., specificity will be examined against the global culture collection and comparisons will be made with genetic groupings.

(2) Optimize laboratory techniques for counting, separation, and obtaining physiological measurements of target species. Techniques for detection, counting, and separation will be perfected for different *Alexandrium* species. Once separation methods have been optimized, species-specific physiological measurements can be performed.

(3) Application of previously developed detection and separation techniques for studying natural bloom populations and dynamics from field samples. Methods optimized in phase 2 above will be applied to blooms of *Alexandrium* in coastal waters of the Gulf of Maine.

**Status:** To date, flow cytometric techniques have been used to detect *Alexandrium* in spiked field samples using immunofluorescence. A monoclonal antibody was used to separate and enumerate these cells from field samples. Cells were positively identified by flow cytometry and sorted. Subsequently, the sorted cell population was observed and confirmed using epifluorescent microscopy. Antibody labeled *Alexandrium* can thus be separated from mixed phytoplankton assemblages. Optimization of this method and others previously described will continue to be examined and will be applied to natural bloom populations in coastal waters.

# ELECTRO-REDUCTIVE DEHALOGENATION OF PRIORITY POLLUTANTS IN AN AQUEOUS SYSTEM AT PALLADIZED GRAPHITE CATHODES

**Richard W. Presley**
Department of Chemistry
New Mexico Highlands University

**Goal:** To investigate an electroreductive process for possible remediation of ground water contaminated with chlorinated organic compounds, focusing on optimum catalyst loading, parameters influencing the efficiency of the process, fate of the substrate, and longevity of the electrode.

**Rationale:** Elimination of both point and non-point sources of pollution will prevent further degradation of our aquifers. In addition to enforcement of these strict control measures, remediation strategies need be developed and implemented. Chlorinated organic compounds are ubiquitous, constitute a high percentage of priority pollutants, and include PCE, TCE, PCBs, chloroform, and carbon tetrachloride. These compounds are good candidates for reductive remediation due to their prevalence, toxicity, carcinogenicity, and recalcitrance to oxidative and bio-remediation schemes. Initial data suggest that palladized (coated with palladium) cathode surfaces can be highly effective in the destruction of these species, resulting in complete dehalogenation of the substrate.

**Approach:** The electro-reduction process entails addition of electrons to the substrate and conversion of bound chlorine to chloride ions, with the organic substrate picking up protons to replace the lost chlorine. Bench-scale laboratory studies are being undertaken in batch and flow-cell modes. A potentiostat or galvanostat is utilized to provide either constant potential or current. Substrate, degradation product, and chloride ion concentrations are monitored. Dilute solutions of carbon tetrachloride, chloroform, and chlorobenzenes are made in ultrapure deionized water containing 50 mM buffer. The data provide for the determination of fate, rate, and efficiency of the process. The electrode surface is characterized, and parameters such as pH, catalyst loading, oxygen presence, and initial substrate concentrations are varied and correlated to the system performance.

**Status:** Initial results have shown that carbon tetrachloride is converted to 80% methane and 20% chloroform at good efficiency. In experiments with low initial concentration of carbon tetrachloride (70 ppm), conversion to methane predominated, with chloroform yield as low as 1%. Current studies are underway to eliminate uncertainty in these numbers, more fully characterize catalyst loading and aging correlation, and investigate the degradation of chloroform. Additional systems to be included in the study are PCE, TCE, and PCBs. This study is scheduled for completion around August 1997.

# CONFIDENCE INTERVALS ON VARIANCE COMPONENTS IN MIXED LINEAR MODELS

**Kathleen Purdy**
Department of Statistics
Oregon State University

**Goal:** To develop methods for constructing confidence intervals on components of variance in mixed experimental design models, particularly for individual and linear combinations of variance components in unbalanced mixed linear models.

**Rationale:** Many statistical questions are concerned with measuring variability in data. For instance, in analyzing longitudinally and spatially correlated data, common in environmental studies, it is necessary to determine the sources and magnitudes of variation. To thoroughly understand the components of variance, researchers require both point and interval estimates of the variance components. A number of methods have been proposed for constructing confidence intervals on components of variance in balanced mixed models. However, in unbalanced cases, these methods can yield confidence intervals which are inconsistent with the stated confidence level. Hence, there is a need for new confidence interval procedures which produce consistent intervals and can be applied generally.

**Approach:** A method is being developed that is likely to produce intervals generally consistent with the stated level and often narrower than intervals constructed by other methods. In particular, a class of statistics which are defined for any mixed linear model has been developed. Under certain conditions, the distribution of these statistics is exactly chi-squared and they may be well approximated by a chi-squared distribution when conditions are not satisfied. This research proposes that these statistics be used to construct confidence intervals on individual variance components and linear combinations of variance components in any mixed linear model. The recommended method is based on a modification of the Cornish-Fisher expansion.

A Monte Carlo simulation study will be performed to compare the proposed method to other recommended procedures. A wide range of designs will be considered. The performance of the interval procedures for each design will be evaluated by its coverage probability and average interval width. Finally, data from various biological studies will be obtained to illustrate the proposed method.

**Status:** A procedure for constructing confidence intervals on variance components in unbalanced completely nested mixed linear models has been developed. Preliminary simulation studies show that the proposed method is generally consistent with the stated level and often narrower than intervals constructed with other recommended methods. Methods for unbalanced non-nested mixed linear models are currently being investigated. Dissertation write-up is expected to be complete by June 1998.

# ELUCIDATION OF THE SIGNALING PATHWAY IN PLANT DEFENSES

**Debra Rate**
Department of MCD Biology
University of Colorado, Boulder

**Goal**: To understand the mechanisms of the signaling pathway leading to the plant defense response against pathogens, with particular focus on the genetic components involved.

**Rationale**: The elucidation of the defense response pathway may lead to the rational design of disease and stress resistant agriculturally important plants. The use of resistant plants would lead to a reduction in chemical use and thus bring the use of pesticides to a sustainable level.

**Approach**: The plant defense response involves the coordinate regulation of many genes. Only a few genes implicated in this regulatory process have been identified. This research is designed to elucidate additional steps in the regulation of defenses and how these defenses are coordinately activated. Induced defenses are characterized by a set of physiological and cellular changes including localized cell death, cell wall modifications, accumulation of anti-microbial compounds, and the induction of several defense-related genes (*BGL2, PR1, GST1* and *LOX1*). This study focuses on events that occur downstream of the initial signal of pathogen perception, specifically, the pathways leading to induction of both *GST1* and *LOX1* by pathogen infection. *GST1* and *LOX1* have been chosen because they display different kinetics of induction and different modes of regulation during the defense response.

Mutants which show aberrant expression of *GST1* and *LOX1* either in the absence of a stimulus or in response to pathogen infection or other stimuli will be isolated and characterized. Ozone, similar to pathogenic stimulation, induces a subset of the defenses associated with the plant defense response. Using ozone as a stimulus for the defense response in screening strategies will indicate whether the signaling pathways leading to the expression of defense genes from pathogen recognition and ozone are distinct or manifested as a single intersecting pathway. Transgenic *Arabidopsis thaliana* plants harboring reporter gene fusions are used to facilitate mutant identification. These gene fusions contain the promoters of *GST1* and *LOX1,* respectively, fused to the reporter genes ß-glucuronidase *(GUS)* or luciferase *(LUX).* The advantage to using transgenic plants harboring reporter fusions is that mutant screening is relatively simple, and many potential mutants can be screened simultaneously. The use of reporter genes will allow identification of mutants in the absence of phenotypic symptoms or in the presence of subtle phenotypes.

Characterization of *pGST1-GUS* expression has shown that the temporal and spatial expression of *GST1* differs from previously characterized defense gene expression *(BGL2).* For example, *pGST1-GUS* expression is localized to the site of infection, whereas *pBGL2-GUS* expression is found on the peripheral edges of the infection zone. The expression of *pGST1-GUS* in the transgenic line is at a high level within three hours post-inoculation with a bacterial strain capable of inducing the defense response pathway in *Arabidopsis thaliana.*

**Status**: Currently, plants grown from mutagenized seed are being screened for mutations which cause constitutive expression of *pGST1-GUS.* Thus far, a number of putative constitutive mutants of *pGST1-GUS* expression have been identified. These putative mutants have expressed high levels of GUS activity at two developmental stages (15 days and 8.5 weeks). Screening of transgenic *Arabidopsis thaliana* plants for mutants displaying non-inducible expression of *pGST1-GUS* will begin shortly. *Arabidopsis thaliana* have recently been transformed with *pLOX1-GUS* constructs and characterization of the *LOX1* expression during the defense response induced by different stimuli will begin soon. Research is scheduled for completion by December 1999.

# CYSTEINE CONJUGATE *S*-OXIDATION BY FLAVIN-CONTAINING MONOOXYGENASES

**Sharon Ripp**
Environmental Toxicology Center
University of Wisconsin-Madison

**Goal:** To determine whether *S*-oxidation is a metabolic pathway (either bioactivation or detoxication) for halogenated alkenyl cysteine conjugates in mammals, and if so, which enzymes catalyze the reaction..

**Rationale:** Cysteine conjugates are intermediates in the metabolism of electrophilic compounds. The cysteine conjugates of the halogenated hydrocarbons trichloroethylene, tetrachioroethylene, *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC), and *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC) are toxic. *S*-Oxidation has been shown to occur with some cysteine conjugates. The purpose of this study was to determine whether *S*-oxidation occurs with DCVC, TCVC, and the cysteine conjugate of allyl halides, *S*-allyl-L-cysteine (SAC). *S*-Oxidation of DCVC and TCVC would result in formation of an α, ß-unsaturated compound that would be expected to be very reactive with nucleophiles (molecules with an affinity for nuclei, *i.e.*, electron-donors) and potentially more toxic than the parent cysteine conjugates. Therefore, *S*-oxidation is a potential bioactivation pathway for DCVC and TCVC.

**Approach: Four cysteine conjugates** were used in this study, DCVC, TCVC, SAC, and *S*-benzyl-L-cysteine (SBC) which had previously been shown to be an *S*-oxidase substrate and therefore was used as a positive control. The cysteine conjugates and their respective sulfoxides were chemically synthesized, and methods were developed for detection and quantitation of the sulfoxides. Rabbit liver microsomes and cDNA-expressed flavin-containing mono-oxygenases (FMOs) were incubated with cysteine conjugates and analyzed for formation of sulfoxides. Sulfoxide reactivity was studied by *in vitro* reaction with the nucleophile glutathione.

**Status:** DCVC, TCVC, SAC, and SBC were found to be *S*-oxidized by rabbit liver microsomes. Studies with inhibitors suggested that FMOs catalyzed the reactions. Results using recombinant FMOs showed that FMO3 was the isoform that catalyzed the *S*-oxidation of DCVC, TCVC, and SAC most efficiently. DCVC and TCVC sulfoxides were very reactive with GSH, whereas the sulfoxides of SAC and SBC did not react with glutathione. Future goals are: (1) to determine FMO3 expression levels in various laboratory animals and in humans in order to find an appropriate animal model, and (2) to study the *S*-oxidation of cysteine conjugates *in vivo* using SAC as a model compound. This research is expected to be completed by September 1998.

# MICROBIAL REMEDIATION OF SOILS CO-CONTAMINATED WITH 2,4-DICHLOROPHENOXYACETIC ACID AND CADMIUM

**Timberley M. Roane**
Department of Soil, Water and Environmental Science
University of Arizona

**Goal:** To investigate whether metal sequestration by metal-resistant microbial populations can reduce metal toxicity within contaminated soil, and whether bioaugmentation with metal-resistant microorganisms can bioremediate co-contaminated soils.

**Rationale:** Co-contaminated sites may prove more difficult to bioremediate due to the nature of the mixed contaminants. While the bioremediation potential of such sites is little understood, it is thought that metals impose a greater stress on microorganisms than do organic contaminants. Specifically, information on how exposure to metal contaminants influences metal-resistance mechanisms and how these mechanisms may enhance the bioremediation potential of contaminated sites is needed. This project will attempt to use microbial metal-resistance to detoxify metals and enhancing degradation. Due to their toxic nature, the two model contaminants for this project are 2,4-dichlorophenoxyacetic acid (2,4-D; organic) and cadmium (Cd; metal).

**Approach:** The overall hypothesis of this study is that stress imposed by a metal contaminant on indigenous soil microbial populations can result in the development of active or directed resistance mechanisms in specific members of the population that may subsequently enhance metal sequestration and organic degradation in co-contaminated soils. Microbial populations will be examined for metal-resistance by monitoring the community response to increasing levels of cadmium in soil slurries. Culturable methods will be used to obtain metal-resistant isolates, and fingerprints of dominant isolates will be taken. Minimum inhibitory concentrations of cadmium for dominant isolates will be evaluated, while polarization and electron microscopies will distinguish between different mechanisms of metal-resistance. Plasmid profile analysis and plasmid curing studies will be used to determine those mechanisms that are plasmid-encoded.

Bioaugmentation studies with metal-resistant isolates will be conducted in cadmium only and cadmium plus 2,4-D contaminated soils. Following bioaugmentation with selected isolates, metal-contaminated and co-contaminated soils will be analyzed over time for available/toxic cadmium concentrations. Culturable isolates from these soils will be subjected to fingerprinting and plasmid profile analyses to determine whether new metal-resistant isolates have emerged through horizontal gene transfer. In the co-contaminated soil, biodegradation rates of 2,4-D will be monitored. If successful, the results of this research will enhance metal sequestration and organic degradation within a co-contaminated soil.

**Status:** Several cadmium-resistant populations have been isolated and characterized. Current studies involve the bioaugmentation of metal and metal-organic co-contaminated soils with cadmium-resistant isolates employing differing mechanisms of resistance to determine the influence on metal availability and toxicity and on organic degradation. This project is expected to be completed within the next two years.

# THE MECHANISMS OF ELEMENTAL MERCURY FORMATION IN SEAWATER

**Kristofer R. Rolfhus**
Department of Marine Sciences
University of Connecticut

**Goal:** To establish the principal biological and chemical controls responsible for the conversion of dissolved ionic Hg (mercury) to gaseous elemental mercury (Hg°) in coastal waters, and to examine the relative importance of Hg° production and its subsequent evasion from surface waters in the overall Hg cycle in a coastal marine system, Long Island Sound (LIS).

**Rationale:** Mercury biogeochemistry has global and regional significance because it is a human health hazard (as the neurotoxin methyl Hg), readily bioaccumulates in aquatic food webs, is primarily anthropogenically derived, and is extremely mobile in atmospheric and aquatic environments. This research attempts to determine mechanisms and reaction rates for the production of Hg°, which is a significant fraction of the overall Hg cycle. Such information will aid in modeling and marine management decision-making.

**Approach:** This research attempts to combine measures of spatial/temporal Hg° distribution with laboratory experiments in order to elucidate mechanisms and reaction rates. Elemental mercury (a dissolved gas) was measured by aspirating a 2 liter sample with an inert gas, while collecting the stripped Hg on a gold amalgam trap. The Hg° was then thermally desorbed from the trap and analyzed. Hg° distributions for LIS were determined seasonally on four separate cruises, while the Connecticut River Estuary was sampled to investigate the hypothesis that estuaries are important sites of "reactive" Hg formation. Bottle incubation experiments (where $Hg^{+2}$ standard is spiked into seawater samples) were performed using coastal seawater collected from the University of Connecticut Marine Science Campus (UCMS). These experiments tested the effects of varying sunlight, temperature, Hg speciation, size fractionation, dissolved organic material, and metabolic inhibition. Surface water concentrations were applied to a gas exchange model to estimate the flux of Hg° from LIS relative to other Hg imports and exports.

**Status:** To date, all field sampling cruises have been completed. A preliminary budget for LIS has been completed and suggests that the gas efflux to the atmosphere is 25-35% of the total Hg input. Laboratory experiments at UCMS are ongoing and suggest that heterotrophic bacteria are responsible for reducing $Hg^{+2}$ to Hg°, but that Hg speciation (complexation with organic matter) is the ultimate control on the availability of "reactive" Hg and thus reduction rate. This work is planned for completion by the end of Summer 1997.

# INTERACTIONS BETWEEN COBALT AND MARINE PHYTOPLANKTON

**Mak Saito**
Joint Program in Chemical Oceanography
Woods Hole Oceanographic Institute and MIT

**Goal:** To determine the influence of trace metals in oligotrophic (nutrient-deficient) regimes on prokaryotic phytoplankton populations, with a particular focus on the interactions between cobalt chemical speciation, organic complexation, and the nutritive requirements of the abundant phytoplankter *Prochlorococcus marinus*.

**Rationale:** Of all the major ocean basins, the Atlantic is the most influenced by anthropogenic perturbations. While very little data exist for trace metal concentrations in the Atlantic (especially for cobalt), recent evidence shows significant increases in metals from human activity (*e.g.*, lead from leaded gasoline). Moreover, some research suggests that acid rain deposition and land use are increasing the amount of cobalt being leached from soils and influencing coastal ecosystems. Finally, changes in global climate can also contribute to oceanic metal concentrations by increasing the dust flux from arid regions.

The photosynthetic cyanobacteria *Synechococcus* and *Prochlorococcus* are important to productivity in the oligotrophic Atlantic: *Prochlorococcus* alone has been estimated to account for 25% of total annual primary productivity in the Sargasso Sea. The limited data on cobalt concentrations in the Sargasso Sea show very low concentrations of total cobalt, ranging from 20 pM to 150 pM (150 x $10^{-12}$M). Recent research shows that *Synechococcus* has an absolute requirement for cobalt, and this study shows that the same is true for the recently discovered *Prochlorococcus*.

**Approach:** Trace metal clean culture experiments and bottle incubations are being conducting in the field to determine the requirements of *Prochlorococcus* and *Synechococcus* with respect to variations in free cobalt and organically complexed cobalt. Field work is being conducted aboard the *RN Oceanus* in the Sargasso Sea. The chemical speciation of cobalt in the surface waters will control its bioavailability, albeit in as yet unknown ways. Therefore, a rapid scan electrochemical cathodic stripping voltametry method has been adapted for conducting sensitive cobalt speciation measurements. The chosen analytical method utilizes nitrite catalyzed reduction of the Co(II)-DMG (dimethylglyoxime) complex and a scan rate of 7000 mV/sec at the surface of the mercury drop.

**Status:** The electrochemical method development is well underway: currently, there is an 8pM detection limit for cobalt and 50 pM blank. Moreover, this work has shown that *Prochlorococcus* has an absolute requirement for cobalt that is within the range of environmental concentrations. Preliminary field evidence suggests the possibility of strong organic complexation in surface Sargasso Sea waters; and preliminary laboratory data suggest the possibility that a Sargasso isolate of *Prochlorococcus* secretes extracellular cobalt chelators that are enhancing growth under cobalt-limited conditions. This has been the basis of pre-thesis work; the second year of five is now being completed. A thesis proposal to continue work on this project or to begin a coastal trace metal-phytoplankton assessment project will be written this summer.

# REGULATION OF GENE EXPRESSION BY THE HEAT SHOCK TRANSCRIPTION FACTOR IN RESPONSE TO ENVIRONMENTAL STRESS

**Nicholas Santoro**
Department of Biological Chemistry
The University of Michigan

**Goal:** To gain a better understanding of the mechanisms by which heat shock transcription factor (HSF) is able to regulate gene expression in response to a variety of environmental stresses.

**Rationale:** The elevated expression of genes encoding heat shock (stress) proteins and molecular chaperones has been shown to protect cells against a broad range of toxic conditions, including oxidative stress, thermal stress, and heavy metal exposure. HSF binds to specific DNA sequences, called heat shock elements (HSEs), in the promoter region and activates expression of a number of genes critical for cell response to environmental stress. Little is known about the role HSEs have in regulating gene transcription in response to environmental trauma. The yeast metallotheionein gene (CUP1) is an excellent model promoter because expression of this gene is activated by heavy metal exposure through the transcription factor ACE1 and by both thermal and oxidative stress through the transcription factor HSF. HSF has been found in all eukaryotic (nucleated) cells, and the DNA-binding domain of HSF has been shown to be very highly conserved among all species. Studying the interaction of the yeast HSF with a model yeast promoter can show whether the HSEs of human promoters similarly influence the ability of human genes to respond to a variety of environmental stresses.

**Approach:** Yeast provides an excellent system for study of the activation of gene expression because powerful genetic and molecular biology techniques are available to manipulate it. A series of CUP1 promoter/lacZ gene fusions has been constructed to study the regulation of CUP1 by HSF in response to both thermal and oxidative stress. These CUP1/lacZ fusions will help in determining what sites in the CUP1 promoter are necessary for *in vivo* activation of CUP1 by HSF in response to thermal stress and oxidative stress. One HSE in the CUP1 promoter essential for activation of CUP1 by HSF in response to thermal stress and oxidative stress has been mapped. However, it is not clear whether additional HSEs in the CUP1 promoter contribute to the ability of this gene to be regulated by HSF. Results from the CUP1/lacZ fusion studies suggest that there is an additional HSE in the CUP1 promoter which contributes to the complex mechanism of activation of this gene by HSF. A system has been developed to produce purified HSF from *E. coli.* This purified HSF is currently being used for *in vitro* DNA binding studies to determine whether this novel second HSE in the CUP1 promoter is actually bound in a specific manner by HSF. The high degree of conservation in the DNA-binding domains of HSFs suggest that studies of the interaction of yeast HSF with the CUP1 promoter HSEs will provide a useful model for how HSEs in the promotors of human genes may influence activation by the human HSF.

**Status:** HSF is currently being purified for use in *in vitro* DNA-binding studies to determine whether HSF actually binds the novel second CUP1 HSE. Studies will be complete in September 1998.

# COPPER TOLERANCE OF BACTERIAL POPULATIONS IN THE PHYLLOSPHERE

**Heather J. Scheck**
Department of Botany and Plant Pathology
Oregon State University

**Goal:** To quantify the variability and distribution of copper-tolerant populations of the phytopathogenic bacterium *Pseudomonas syringae* on the phyllosphere (leaf areas) of containerized woody plants grown in commercial ornamental nurseries.

**Rationale:** Because of the heavy use of copper-containing bactericides for disease control in commercial ornamental woody plant nurseries, copper tolerance is increasing in populations of the bacterial blight phytopathogen (plant parasite), *Pseudomonas syringae.* This increase is in both the size of the copper-tolerant pathogen populations and the quantity of copper ions that can be tolerated. Despite the strong selection pressure for tolerance to copper in these environments, sensitive individuals and individuals with only moderate levels of tolerance have been consistently isolated from the phyllosphere of diseased woody plants. Because these populations are persistent in environments of high bactericide use, there may be a fitness cost associated with maintaining a copper-tolerant phenotype. Withdrawing copper bactericide applications could lead to a decrease in tolerant populations over time.

**Approach:** Bacteria were isolated from the phyllosphere of two species of woody plants: *Acer palmatum* (Japanese Maple) and *Syringa vulgaris* (common Lilac). Samples were collected at random within large blocks of containerized plants from five commercial nurseries and one landscape planting of lilacs in the Willamette Valley, Oregon. Bacteria identified as *Pseudomonas syringae* were evaluated for copper tolerance on differential media amended with copper sulfate. Bacterial populations most closely followed a lognormal probability distribution and were log-transformed to allow conformance to the assumptions of the analysis of variance procedure. There were no significant differences in the numbers of copper-sensitive or copper-tolerant bacteria isolated from maple or lilac. No copper-tolerant bacteria were isolated from landscape-planted lilacs which had not been exposed to copper-bactericides for more than twenty years. There were significant differences in the number of copper-tolerant bacteria isolated from proximal commercial nurseries, which may be a reflection of copper bactericide usage. Strains isolated from a single disease lesion also showed variability in levels of copper-tolerance. Possible explanations include a high frequency of back mutations resulting in the loss of copper tolerance, or several individual strains with different levels of tolerance co-initiating what appeared to be a single lesion.

**Status:** This project is one chapter in a doctoral dissertation and is essentially complete. Dissertation defense is planned for completion by August 1997.

# DIETARY METAL UPTAKE BY THE ESTUARINE AMPHIPOD *LEPTOCHEIRUS PLUMULOSUS*

**Christian E. Schlekat**
Department of Environmental Health Sciences
University of South Carolina

**Goal:** To determine the bioavailability of metals associated with oxidized surficial sediments through dietary ingestion by estuarine benthic invertebrates, focusing on whether metals associated with labile sediment features (biofilms and microalgae) are more efficiently transferred to organisms than metals associated with refractory sediment features (lignins, tannins, *etc.*).

**Rationale:** In general, there are two pathways by which aquatic organisms can adsorb and subsequently bioaccumulate contaminants: (1) across permeable membranes (gills) for dissolved metals, and (2) through dietary ingestion for particulate-associated metals. The relative importance of these two uptake routes is poorly understood; however, dietary uptake presumably plays a major role for organisms living in benthic (bottom) portions of estuaries because metals are strongly associated with particulate matter in these environments and because many estuarine invertebrates selectively ingest particulate food items from surficial and suspended sediments.

**Approach:** The efficiency by which the estuarine amphipod (a type of crustacean) *Leptocheirus plumulosus* assimilates metals associated with particulate matter representative of a broad spectrum of food quality will be measured. Because the relative importance of dietary accumulation of metals associated with a wide range of potential sediment features is dictated in part by the metal-binding characteristics of these features, the gamma emitters $^{109}Cd$ (cadmium) and $^{51}Cr$ (chromium) were first used to measure the adsorption of Cd and Cr to a variety of model particles. In initial experiments, these particles included (1) silica particles with an organic coating comprised of bacterial extracellular polymeric substances (EPS); (2) sterilized estuarine sediment, and two species of phytoplankton; (3) the diatom *Phaeodacylum tricornutum;* and 4) the chlorophyte *Dunaliella tertiolecta*. The efficiency at which *L. plumulosus* assimilated Cd and Cr associated with each of these particles was then measured. Results highlight the potential importance of EPS in the fate and transport of metals in estuarine systems. First, EPS-coated particles rapidly and strongly bind Cd and Cr across a salinity gradient. This is important because EPS sediment coatings are ubiquitous within marine and estuarine surficial and suspended sediments. Also, *L. plumulosus* assimilates Cd most efficiently from EPS-coated particles and least efficiently from the phytoplankton.

**Status:** Work will be continued by comparing the results described above with different metals and with additional model particles. Additionally, stable metal isotopes will be utilized in microcosm studies to address in greater detail the potential role of EPS sediment coatings on the bioavailability and geochemical cycling of metals within estuarine sediments.

# AGE-DEPENDENT DIFFERENCES IN CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN FISCHER-344 RATS

**Dan Schoeffner**
Department of Pharmacology and Toxicology
University of Georgia

**Goal:** To determine whether aged rodents are more susceptible to the hepatotoxic effects of carbon tetrachloride ($CCl_4$) than their younger counterparts, and if so, to identify the underlying mechanism(s) responsible for these differences.

**Rationale:** $CCl_4$ is a ubiquitous environmental contaminant which exerts its greatest influences on the liver, the kidneys, and the central nervous system. It has been identified at more than 20% of the sites on the Superfund National Priority List, but the actual number of sites contaminated is not known at this time. For any environmental contaminant it is important to determine which segments of the population are most susceptible in order to protect the entire population. It has been hypothesized that the elderly may be more susceptible to chemicals, since the frequency of idiosyncrasies and other adverse drug reactions increases with age. If this rationale holds true for industrial chemicals, the elderly may be considered a subpopulation sensitive to environmental contaminants.

**Approach:** Young (3 months) and old (22-24 months) male Fischer-344(F-344) rats were utilized in this project to identify the influence of age on the severity of acute liver injury. Dosages of 0, 20, 100, and 500 mg/kg of $CCl_4$ were given orally at a time when the liver is most susceptible to injury (3:30-4:00 p.m.). This dosing regime was chosen to maximize the likelihood that subtle differences in susceptibility would be detected. Twenty-four hours post-dosing, all animals were sacrificed, and blood and liver samples were collected. Serum samples were analyzed for alanine aminotransferase (ALT) and sorbital dehydrogenase (SDH) activities, two indicators of parenchymal liver cell damage. Portions of the left hepatic lobe were sectioned for histopathological evaluation of centrilobular necrosis (tissue breakdown) and vacuolar degeneration. Small liver samples were taken from the control animals in order to determine levels of lipid-soluble antioxidants, glutathione (GSH), and GSH-related enzymes. These indices reflect the ability of each age group to withstand hepatic insult. Additional portions of control liver were taken and processed into microsomal suspensions. Enzymatic activity assays were then performed on these samples. Since nearly all $CCl_4$ damage is directly linked with microsomal metabolism of $CCl_4$, it will be necessary to determine if changes in metabolism influence the outcome of $CCl_4$ exposure. Investigation has revealed that differences in sensitivity to acute $CCl_4$ hepatotoxicity exist between young and old male F-344 rats. The older rats exhibited significantly higher serum levels of ALT and SDH and exhibited more severe centrilobular necrosis than did young rats over a wide range of oral dosages. Analysis of CYP2E 1 (the main enzyme responsible for metabolic activation of the $CCl_4$ molecule) activity revealed no difference between the ability of young and old rats to metabolize $CCl_4$. There was also no indication that the ability to withstand the initial toxic insult differed significantly between the ages.

**Status:** Future possible avenues of investigation include comparison of hepatic repair, Kupffer cell activity, or pharmacokinetic studies. Selection will involve careful evaluation of the information collected to date, review of current literature, and feasibility. Research will be completed no later than September 1998.

# ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL FROM WASTEWATER

**Andrew J. Schuler**
Department of Civil and Environmental Engineering
University of California, Berkeley

**Goal:** To provide new reasons for reported inconsistencies with the metabolic model of Enhanced Biological Phosphorus Removal (EBPR, the process by which bacteria in wastewater treatment systems are induced to accumulate excess phosphorus) by clarifying the interactions between the intracellular storage products polyphosphate, glycogen, and poly-ß-hydroxybutyrate (PHB), which are part of this model; to provide reasons for the instability and limits of phosphate uptake of EBPR systems; and to isolate a causative organism of EBPR.

**Rationale:** Removal of phosphate from wastewater protects sensitive receiving waters from algal blooms. Many full-scale wastewater plants currently use EBPR, but due to a lack of understanding of the EBPR mechanism, design and operation of such systems is largely empirical, and they are notoriously unstable. Increased understanding of the role of storage products may give clues as to the limits and reasons for instability of EBPR systems. Isolation of a causative organism(s) of EBPR would eliminate the effects of population dynamics, which have complicated previous attempts to characterize the metabolic model of EBPR.

**Approach:** Two bench-top one-liter sequencing batch reactors have been operated continuously for nearly two years. The reactors operate on a six hour cycle which includes anaerobic, aerobic, settling, wasting, and feeding phases. The reactors have been operated at varying levels of phosphate input ranging from a phosphate-limited system to a phosphate-excess system, and the effect of such variations on intracellular storage products is being monitored. Batch tests are also being conducting to determine the effects of storage products on the rates and limits of substrate (phosphate and carbon) uptake. Isolation of the causative organism(s) of EBPR is being attempted by simulating as closely as possible the conditions EBPR organisms experience in mixed cultures during isolation. Methods for simulation of this environment include the use of sterilized effluent from the mixed cultures as a component of pure culture feed and strict control of the timing of key substrate availability.

**Status:** An inverse correlation between carbohydrate and polyphosphate storage products has been demonstrated, and a correlation with the rate of anaerobic acetate uptake has been observed. These relationships suggests a theoretical limit to the total amount of phosphate that can be stored by EBPR organisms, and the same limit has been verified in the reactors. Results also suggest that limitations of carbohydrate may be responsible for the instability of EBPR systems, which is currently being verified. A pure culture capable of EBPR has not been isolated yet, but this effort continues with modified methods.

# CROSS-CULTURAL NATURAL RESOURCE CONFLICTS: CONTEMPORARY NATIVE AMERICAN WHALING

**Jennifer Sepez**
Department of Anthropology
University of Washington

**Goal:** To examine the various inherent conflicts and potential for cooperative resolutions concerning the protection and management of the California gray whale that accompany the Makah Indian Tribe's proposal to resume hunting this species, recently removed from the endangered species list because of full population recovery.

**Rationale:** Conflicts over natural resource management can be exacerbated strongly when different ethnic and cultural groups are involved. These conflicts present a barrier to conservation efforts worldwide. The basic biological research which most natural resource management agencies are expected to conduct cannot address these issues; yet addressing them is critically important to achieving successful resource conservation. Social and cultural research which seeks to understand the particular dynamics of cross-cultural resource conflicts is necessary in order to develop culturally sensitive public policies that successfully conserve the world's biological capital for the long term.

**Approach:** This study will employ participant observation, ethnohistoric research, cross-cultural comparison, and other anthropological techniques to examine the dynamic interaction between the interested parties: the Makah Tribe, U.S. Federal resource agencies and policy-makers, the International Whaling Commission, conservation groups, and animal rights activists. The material goal is to document and understand how protection and management of the now-recovered California gray whale will develop when the Makah Tribe exercises its treaty-reserved right to hunt whales. The outcome will depend in part on what structures develop at the international level (the International Whaling Commission), the Federal level (NOAA), and the Tribal level. Several questions will be addressed, including (1) What are the key cultural, political, and ecological conflicts between the various parties? (2) Which approaches best help reduce conflict and produce cooperation while still protecting the resource? and (3) How can this serve as a model for other situations in which indigenous populations are claiming or reclaiming resource rights? The negotiation and development of management structures for Inuit whaling in northern Alaska 20 years ago provides a comparative case study in which extreme conflict was transformed into successful cooperation. Because the California gray whale migrates through several national jurisdictions, research also will include interest groups and government policies in Canada, Russia, and Mexico.

**Status:** Research funding began in October 1996 and the project is in its preliminary stages. Research to date has been archival and preparatory. Field work will begin in October 1997 and the project should be completed in June 1999.

# GENETIC ENGINEERING AND ECOLOGY: THE ROLE OF TROPANE ALKALOIDS IN DEFENSE AGAINST INSECT HERBIVORY

**Irene Shonle**
Department of Ecology and Evolution
University of Chicago

**Goal:** To examine the evolutionary ecology of tropane alkaloids, which are found in many plants in the potato family (Solanaceae) in order to provide a mechanistic understanding of the role of plant compounds in natural settings and the evolution of plant chemical defenses.

**Rationale:** Many theories explaining the diversity of plant compounds propose that plants produce these chemicals as defenses against herbivorous insects, but there is little experimental work to demonstrate the validity of this idea. An understanding of the biological effects of alkaloids is a prerequisite to the genetic engineering of potent allelochemicals into plants.

**Approach:** Three strategies will be used to determine the functional importance of the major tropane alkaloids (hyoscyamine and scopolamine) using two closely related members of the Solanaceae family, deadly nightshade and jimson weed. The first approach is to add the alkaloids, both individually and in mixtures, to insect artificial diet in order to ascertain their effects on insect growth and survival. The second strategy is to use a quantitative genetics approach to test whether combinations or amounts of the alkaloids are genetically determined and whether they have a defensive function in natural populations. The final strategy is the most novel and takes advantage of recent biotechnology to insert genes into plants. A gene for a late enzyme in the alkaloid biosynthetic pathway (hyoscyamine 6-ß-hydroxylase) will be over expressed in plants using agrobacterium for the genetic engineering, and tissue culture. This will qualitatively change their alkaloid production (from hyoscyamine to scopolamine). Individuals having these "transgenic" alkaloid profiles will be planted in the field with wild type plants, and the amount of damage and total seed production for plants of both kinds will be measured. This project has implications both for understanding of the evolution of plant chemistry and for the applied management of crops. In particular, findings will help guide genetic engineering of more resistant plants and, in so doing, allow farmers to be less dependent on pesticide application.

**Status:** Insect feeding trials have been performed which show little effect of the alkaloids when incorporated into artificial diet. However, there was a significant detrimental effect on the insects fed tropane alkaloid-bearing plants and of insects fed wild-type *vs.* transgenic plants. Additionally, in field trials over the summer of 1996, the transgenic plants suffered significantly less damage and had higher overall fitness (fruit production) than wild-type plants. A larger field experiment with additional transgenic lines is planned for summer 1998. Some, but not yet all of the planned transgenic lines have been successfully created. The quantitative genetics experiment will be planted out this summer (1997). Research is planned for completion in two years.

# EVALUATION OF SEVEN SAMPLING TECHNIQUES FOR WIREWORMS (COLEOPTERA: *ELATERIDAE*)

**Carol Simmons**
Department of Entomology
Iowa State University

**Goal:** To develop a user-friendly system for sampling for wireworms to be utilized in a sampling program critical for optimizing wireworm management on agricultural land or land exiting the Conservation Reserve Program.

**Rationale:** Since 1985, 36.5 million acres of land in the U.S. have been enrolled in the Conservation Reserve Program (CRP) under ten-year contracts. A goal of the CRP was to reduce soil erosion by taking lands out of production and implementing conservation practices. Grass systems were established on 87% of these lands. Although these grass-dominated systems have satisfied the goals of the program, they have also encouraged the propagation of soil insect pests, specifically wireworms. With the ten-year contracts beginning to expire, CRP likely will return to agricultural production, and potential for wireworm problems is great. Wireworms are pests of corn, small grain, grasses, potatoes, and root crops. Seed damage from this pest can cause substantial stand loss and require subsequent replanting. The need for detecting wireworms before planting is crucial, since there is currently no insecticidal rescue treatment available for infestations.

**Approach:** During 1995 and 1996, seven sampling techniques were examined to aid in developing a farmer- or consultant-oriented user-friendly system of sampling for wireworms to determine field populations. In an intensive sampling program, the soil core (absolute) sampling method was compared to six relative sampling methods (corn/wheat bait, melon bait, potato bait, wire-mesh bait, pheromone trap, and pitfall trap). In an extensive sampling program, the corn/wheat bait was examined for its utility in Conservation Reserve Program habitats. Each method was evaluated for its precision and accuracy in determining populations of wireworms. During 1995-1996, 1,775 wireworms were collected from 4,860 samples. The corn/wheat bait showed the highest level of precision (RV = 9.30) and the highest level of accuracy ($r = 0.89$ $P < 0.01$) in the intensive sampling program. Acceptable levels of precision also were found in the extensive sampling program for corn/wheat baits. The corn/wheat bait was the most cost effective ($0.95 per sampling unit). When examining relative net precision, the corn/wheat bait is the most cost efficient and effective sampling method for determining wireworm populations.

**Status:** This research has been completed, and the goal was successfully accomplished. This sampling program makes it possible to implement pest management tactics against economic populations of wireworms. The implementation of this sampling program will result in insecticidal management tactics related only wireworm populations of economic importance.

# THE KINETICS OF URANYL ION REDUCTION IN AQUEOUS SOLUTION BY SULFATE-REDUCING BACTERIA

**John R. Spear**
Division of Environmental Science and Engineering
Colorado School of Mines

**Goal:** To determine and model the reaction kinetics for the enzymatic reduction of soluble uranium (U[VI]) to insoluble U(IV) by a mixed culture of wild-type sulfate-reducing bacteria (SRB) under a variety of solution conditions, and to analyze and model the surface adsorption effects of U(VI) binding onto the surface of colloidal size SRB.

**Rationale:** Natural and anthropogenic uranium contamination of surface- and groundwater is widespread. Current treatment methodologies are effective but can be prohibitively expensive in treating the millions of gallons of low-level uranium-containing waters. With an understanding of the reaction kinetics for the enzymatic reduction combined with the surface adsorption effects of the cells themselves, a biologically based treatment scheme for the removal of U(VI) from contaminated surface- and groundwater can be developed.

**Approach:** Previous studies have indicated that pure cultures of certain types of bacteria are capable of enzymatically reducing soluble U(VI) to insoluble U(IV), by using the uranium as the terminal electron acceptor. To date, a wild-type, mixed culture SRB colony has been isolated from an inoculated, manure-filled, acid mine drainage treatment tower. This culture was initially isolated on an ethanol minimal media and has since been grown in a continuously stirred tank reactor (CSTR or chemostat) on a lactate minimal media. Kinetic studies are performed in anaerobic batch reactors with variable amounts of washed cells, U concentrations, and solution conditions. At time points of interest, samples are removed for cell count. Experimental conditions are replicated in quadruplicate, and data are then analyzed individually and averaged. Predictive computer modeling of the enzymatic reduction is then developed using EXCEL spreadsheets. The effects of a secondary process, that of uranium sorption to the surface of the bacteria, is being studied across the biologically active pH range 5-9. Preliminary data suggest that there are two dominant surface functional groups for proton (metal ion) adsorption.

**Status:** Initial kinetic modeling of a large amount of experimental data indicates that a mixed order Monod model satisfies the experimental outcome. A manuscript of this information will be submitted for publication in Spring 1997. The next step is a kinetic evaluation by a similar protocol for sulfate metabolism. Concurrently, work is continuing on the surface adsorption studies. Completion of this research is planned for 1998 or 1999.

# CAN MICROBIAL COMMUNITIES IN STREAM SEDIMENTS BE USED TO EVALUATE THE AVAILABILITY AND COMPOSITION OF DISSOLVED ORGANIC CARBON IN STREAM ECOSYSTEMS?

**William Sobezak**
Ecology and Systematics
Cornell University

**Goal:** To examine the utility of using microbial responses to evaluate the bioavailability of dissolved organic carbon (DOC) in transport in streams and rivers.

**Rationale:** Microbes in stream sediments are thought to be sensitive to changes in the flux of dissolved organic carbon (DOC). Although DOC is thought to influence the abundance and activity of bacteria across aquatic ecosystems, the availability of a DOC source can be difficult to assess due to the complexity and diversity of its constituents. In addition, the majority of DOC in transport is frequently refractory or difficult for microbes to assimilate and represents the unavailable pool of DOC. Hence, traditional analytical methods for isolating specific carbon compounds or characterizing "operational classes" of compounds cannot adequately assess the availability or labile (readily changed by chemical or biological means) fraction of a DOC source.

**Approach:** Three questions will be addressed: (1) What fraction of stream DOC is available for microbial assimilation and metabolism? (2) What are the chemical characteristics of labile or bioavailable DOC? and (3) What are the most sensitive and informative microbiological methods for characterizing microbial responses to natural variation in bioavailable DOC? Responses of microbial communities may be used to evaluate the availability and labile fraction of DOC. These responses may include: (1) "bulk community" responses (changes in abundance or activity); (2) "phenotypic" responses (changes in enzyme activity or carbon acquisition); (3) "genotypic" responses (changes in microbial composition). Research will focus on microbial responses along selected sub-surface flowpaths (both natural and experimental) in which the initial DOC concentration and rate of loss varies. These environments (stream hyporheic zones) are ideal for such an examination because they maximize DOC contact with bacteria and allow a discreet source of DOC to be examined along a utilization continuum.

**Status:** Future work will examine the extent that bacterial community structure covaries with functional response to gradients of dissolved organic carbon. Research is nearly complete and thesis defense is scheduled for spring of 1997.

# AMERICAN INDIAN WATER RIGHTS AND THE ENVIRONMENT

**Alex Steenstra**
Department of Economics
University of Utah

**Goal:** To develop an interdisciplinary approach that facilitates efficient, equitable, and environmentally sound allocation and application of Indian water for the purpose of creating new opportunities leading to sustainable economic development and improved water uses in the West.

**Rationale:** Water is essential for the development of reservation economies, but non-Indians fear that the recent validation of Indian water rights may halt non-Indian economic development. The potential Indian water claims have been estimated at nearly 46 million acre-feet per year. While non-Indian water policy is slowly incorporating contemporary values, including the emphasis on environmental uses, Indian water policy is still based on the nineteenth century objective of irrigated agriculture. In particular, the legal quantification method to determine the extent of Indian water is based on the economic feasibility of diverting and storing water for agricultural purposes only. Interstate compacts, in addition, severely limit Indian water applications.

**Approach:** It is the premise of this research that the integration of the contemporary principles of efficiency, equity, and ecology into Indian water policy will generate prospects for resolving the conflict and lead to sustainable development. The principle of efficiency promotes the allocation of water to satisfy a broad range of demands; the principle of equity stresses that all interests are allowed to influence water use decisions according to their stake in the outcome; and the principle of ecology ensures environmentally sound water uses based on the science of ecology. The proposed approach, therefore, is based on an integration of sound economic reasoning into a necessarily complex interdisciplinary forum. These efforts have generated the Indian Water Rights Impact Statement (IWRIS) as an alternative method to determine the quantity and use of Indian water. This method evaluates monetary and non-monetary costs and benefits in an effort to consider relevant concerns. An IWRIS seeks the active participation of affected parties to encourage agreement and the use of reliable knowledge to understand the problems involved and recommend possible solutions through a five step process of scientific assessment, risk analysis, public involvement, implementation, and reassessment. In addition, an IWRIS employs the minimal displacement criterion, that is, it avoids unduly disruptive changes in the environment and lives of people.

**Status:** The details of the IWRIS are nearly completed but its application to case studies has proven to be more difficult than originally anticipated. Little published information is available and efforts to obtain information directly from Indian tribes and other sources have mostly been frustrated, but some promising contacts have recently been made. This research, therefore, is expected to be completed in the summer of 1998.

# THE INFLUENCE OF $C_4$ PHOTOSYNTHESIS ON THE CONCENTRATION AND ISOTOPIC COMPOSITION OF ATMOSPHERIC $CO_2$

**Chris Still**
Department of Biological Sciences
Stanford University

**Goal**: To obtain quantitative estimates of the influence of $C_4$ photosynthesis on the concentration and isotopic (carbon and oxygen) composition of atmospheric $CO_2$.

**Rationale**: This research will address the uncertainty inherent in partitioning the observed carbon sink between the land and the ocean. It will also improve understanding of the role of tropical ecosystems, particularly savannas where $C_4$ plants reach their greatest extent, in the global carbon cycle.

**Approach**: This research combines modeling and field work. The land surface model SiB2 is being used to obtain refined estimates of the proportion of global GPP undertaken by $C_4$ plants. This is accomplished by combining a vegetative cover map derived from satellite data with a climate-space index that predicts whether or not a given grid cell should contain $C_4$ plants. In principle, this combination allows a determination of the fraction of production due to the $C_4$ photosynthetic pathway within a given grid cell. In addition, the influence of these plants on the concentration and isotopic composition of atmospheric $CO_2$ will be investigated by coupling the surface fluxes to a tracer-transport model. The field work will hopefully be a part of the upcoming Large-Scale Biosphere Atmosphere Experiment in Amazonia (LBA), as this offers the ideal venue in which to pursue the kind of research needed for this project. Specifically, flux measurements and the associated isotopic discriminations during the processes of photosynthesis and respiration in the Amazonian savanna (also called the cerrado) will be obtained. A modified branch bag system will be used on selected ($C_3$) tree species and a similar system on ($C_4$) grasses for simultaneous gas exchange measurements and isotopic discrimination determinations during carbon uptake. Similar measurements will be made during carbon release using a modified soil respiration chamber. In order to calculate the ecosystem discrimination against $C^{18}OO$ during these processes, it will be necessary to determine the oxygen isotope composition of the various water pools with which $CO_2$ molecules react, namely the leaf water and soil water. Finally, to relate surface fluxes and isotopic discriminations to larger (basin)-scale processes, this research will be coordinated with that of other groups making similar measurements from nearby towers and from aircraft.

**Status**: The modeling component of this research is well underway, and fieldwork is scheduled to begin later this year. Research should be completed by the summer of 1999.

# MECHANISMS FOR POPULATION EXTINCTION IN SOUTHWESTERN *AQUILEGIA*

**Allan E. Strand**
Department of Biology
New Mexico State University

**Goal:** To demonstrate what studies of effective population size in plant populations can reveal about current and historical processes that give rise to current population distributions.

**Rationale:** Since the end of the Pleistocene, average moisture levels in the deserts of the Southwestern United States have declined. Understanding how this drying trend has historically affected plant populations may allow distinction between short-term and long-term climate change effects in plant populations.

**Approach:** Patterns of genetic diversity in several *Aquilegia* populations in the Southwestern United States are being assessed, and effective population sizes are being estimated using both a demographic approach and a genetic approach. *Aquilegia* is restricted to high moisture sites in this region. As a result, regional changes in moisture level should affect the number of these populations. At least two questions are relevant to the *Aquilegia* system: (1) Are populations declining in number? and (2) If such a decline is occurring, does it result from drying trends? The first question can be answered, at least in part, by fossil evidence. The presence of packrat midden (dung) fossils in the southwest provide us with a picture of vegetation change for the past 40,000 years. This picture suggests that current *Aquilegia* populations exist in refugia (*i.e.*, small areas that escaped significant changes which occurred in the larger area) at high elevations and that historically they were probably distributed more widely throughout lower-elevation watersheds. Under this refugium scenario, there are two possibilities regarding migration and the fate of populations: (1) migration links populations, permitting continued recolonization of extinct refugia, or (2) there is no migration among taxa and as each population goes extinct, the entire system ratchets towards extinction. Estimates of effective population sizes and genetic similarity among populations provide the data needed to distinguish between these possibilities. Even in the total absence of migration, populations that were linked in the past but that have retreated into refugia will resemble each other genetically due to "inherited" genetic similarity. The rate at which that similarity will decay is determined by the effective population size. With estimates of effective size and genetic similarity, it is possible to ask whether or not the current *Aquilegia* system is linked by ongoing migration.

**Status:** The analysis outlined above for the chloroplast genome in *Aquilegia* has been completed. The non-recolonization model fits these data well. Currently, research is progressing along three fronts: (1) the study is being expanded to include information from the nuclear genome; (2) demographic effective size estimates are being refined by adding more years of demography data; and (3) sequences from the nuclear genes required to perform the genetic effective size estimates are being obtained. The data collection phase of this research is planned for completion by mid-summer 1998.

# BIOFILTER TREATMENT OF LANDFILL GAS

**Denise G. Taylor**
Dept. of Geography and Environmental Engineering
The Johns Hopkins University

**Goal:** The goal of this research is to assess the effect of high concentrations of methane and carbon dioxide (typical primary components of a landfill-generated gas) on biofilter treatment of vapor-phase chlorinated solvents. Questions to be addressed are: (1) Can a zero-valent iron (abiotic) pretreatment successfully reduce the level of halogenation in gaseous polyhalogenated solvents? (2) What factors effect the reaction products? (3) Are the gaseous products of the zero-valent pretreatment stage amenable to biodegradation in a biofilter? and (4) Can characteristics of a plug-flow regime in the biofilter be used to successfully treat a mixture of contaminants in the gas waste stream?

**Rationale:** Current air quality regulations will require certain landfills to collect and treat gases generated in the decomposing waste pile. One option for compliance is to reduce non-methane organic components of the gas stream by 98%. These components are often present at very dilute concentrations, for which biodegradation may be beneficial.

**Approach:** A landfill gas is simulated by bubbling a methane/carbon dioxide mixture through the contaminant (as a pure liquid) of interest. For studies of the zero-valent iron stage, <100-mesh iron is acid rinsed, washed with argon-sparged water followed by acetone, and dried under argon. The effect of moisture, carbon dioxide, etc., on the zero-valent iron stage is investigated in batch bottles of 160 ml. Known mass of iron and glass beads are added to the bottles under anaerobic conditions. The solvent-laden gas is purged into the bottles, which are then sealed with Teflon-faced butyl stoppers. In flow-through column studies, the solvent-laden gas is directly attached to a glass-column reactor packed with sand or glass beads and iron. Controls (identical preparation, but omitting one of the components of iron, carbon dioxide, or solvent, for example) are also conducted as appropriate. Solvent parent compounds and products, as well as carbon dioxide, are measured. Molecular genetics techniques such as gene probing are being investigated for analysis and possible quantification of bacterial DNA or its activity to assist in characterizing the biofilm population.

**Status:** The effect of moisture on the zero-valent iron pretreatment stage has been investigated. Major products have been identified and quantified. Additional work is needed on investigating the incorporation of $CO_2$ into products, and on the contribution (if any) of methane to the reduction of parent and intermediate compounds. The immediate next step is further work to establish appropriate protocol for measurement of biological activity or viability in a biofilter reactor. Following this, the majority of research effort will be spent on the biofilter component. At this point in time it appears that a plug-flow regime may have to be simulated or a different approach taken: lab-scale efforts to establish plug-flow have been difficult due to rapid diffusion of the contaminants within the gas phase.

# DEVELOPMENTAL EFFECTS OF 17-α ETHINYL ESTRADIOL

**Kristina Thayer**
Department of Biological Sciences
University of Missouri, Columbia

**Goal:** To elucidate low-dose effects resulting from prenatal exposure to ethinyl estradiol, a synthetic estrogen commonly used in oral contraceptives.

**Rationale:** Ethinyl estradiol can serve as a positive control to which environmental chemicals can be compared to evaluate their estrogenic activity. Traditionally, diethylstilbestrol (DES) has served this role. However, ethinyl estradiol is currently more clinically relevant as it is used in many oral contraceptives (OCs). Between 2-5% of women continue to take OCs during undetected early pregnancy. This experiment will enhance knowledge of the developmental effects of low dose exposure to estrogenic compounds.

**Approach:** Pregnant mice were dosed on days 0-17 of pregnancy with ethinyl estradiol, (0.2, 2, 20, and 200 ng/kg, respectively). These doses include those in the range of many OCs and below. Male and female offspring are then examined in adult life for responses believed to be influenced by prenatal estrogen exposure. These response include behavioral (wheel running activity), reproductive, and hormonal endpoints. In the male, reproductive organs were collected and daily sperm production, prostate weight, and androgen receptor levels analyzed. In the female, at 5-6 months of age, animals were implanted with estradiol capsules, and uterine weight, uterine estrogen receptor levels, and luteinizing hormone levels were measured to see if prenatal exposure had modified the adult animals' response to estradiol.

**Status:**

1. Ethinyl estradiol: All tissues have been collected. However, several assays still need to be completed (*e.g.*, androgen and estrogen receptor assays). To date, in the male offspring, significantly decreased daily sperm production have been observed in animals collected at day 50, but not in those collected at 5 months. Increased prostate weight (a sensitive marker for prenatal estrogen exposure) at both day 50 and 5 months has also been found. In the females, a trend for altered responses to estradiol in adult life has been observed, as measured by luteinizing hormone and uterine weight.

2. Estrogen receptor knockout (ERKO) mice: These animals can serve as a model to assess effects of environmental chemicals not mediated via the major estrogen receptor (ERα). However, before environmental chemicals can be administered to these animals, their responses to synthetic estrogen need to be assessed. Thus, ERKO mice have been dosed with a range of low doses of DES. These animals have been generated and in the next few months, behavioral, cardiovascular, reproductive, and hormonal responses will be measured. Dependent on the results of this experiment, these animals may be dosed with bisphenyl A starting in the summer of 1997.

# BIOGEOCHEMISTRY OF ARSENIC AND ITS EXTRACTION FROM SOIL

**John E. Thomas**
Soil and Water Science Department
University of Florida

**Goal:** To explore potential effective means of remediating arsenic-contaminated soil through extraction, mobilization, or volatilization of arsenic.

**Rationale:** In southern states including Florida, one anthropogenic input of arsenic into soils can be attributed to the extensive use of cattle dipping vats from 1906 to 1962 for the purpose of tick eradication. Once a year, contents of the vats were discarded by dumping the solution onto the adjacent soil. Presently, the main remediation technique for these soils is excavation and disposal in a landfill. With the dwindling number of landfills, it will become more economical to practice in-situ removal of the arsenic from the soil.

**Approach:** The first area of investigation involves chemical complexation and extraction of the arsenical oxyanions (oxygenated negative ions) by an aqueous solution of sodium dodecylsulfate mixed with a highly positive-charged macrocycle (compound containing a ring consisting of ≥15 atoms) synthesized from vitamin $B_1$. The ability to extract arsenic coupled with the expected biodegradability of the organic extractants make this system a strong candidate for classical pump-and-treat remediation or other soil flushing/washing procedures. The second thrust of this study involves the biological volatilization of arsenic. Field studies are conducted to assess volatilization rates off the soil surface using soil gas flux collection boxes. The subsurface flux is also determined using depth probes to collect arsenical gases onto activated charcoal. Laboratory studies are conducted to find arsenic volatilization rates under various oxic (oxygenated) conditions for soil and for mineral culture media inoculated with microorganisms isolated from these contaminated soils. This project consists of six distinct phases: 1) site identification; 2) quantification of the amount and location of arsenic in the soil; 3) evaluation of the chemical extraction procedure of arsenic from the different soil types; 4) testing of the soil for biological volatilization of arsenic; 5) isolation and testing of microorganisms responsible for arsenic volatilization; and 6) determination of the rates of arsenic transformation to volatile compounds by these microorganisms under various oxic conditions.

**Status:** The complex/surfactant extraction procedure has been tested and found to be capable of extracting arsenic with high efficiency (>80%) from both sandy and clayey soils. However, as the amount of clay increases, the amount of macrocycle required for comparable extraction efficiency increases as well. The volatilization of arsenic has been found to occur in the subsurface soil. No volatile arsenic was measured off the surface of the soil. A *Fusarium* species has been isolated and identified as the causative agent at one contaminated site. Influence of various oxygen levels on arsenical gases from contaminated soil and from fungus in a mineral media has been studied. Ground penetrating radar was used at one site to elucidate the subsurface structures that influenced the shape of the contaminant plume. An arsenic plume is being delineated at a second site. Hopefully, this research project will be completed within the next year.

# INVESTIGATION OF THE EFFECT OF MEMBRANE PROPERTIES ON ION TRANSPORT AND SELECTIVITY ACHIEVED WITH FIXED SITE CARRIER MEMBRANES

**Kristin Thunhorst**
Chemical Engineering Department
University of Colorado, Boulder

**Goal:** To understand how ion transport through membranes is affected by structural and material carrier membrane properties, with the aim of developing predictive models and relationships to better understand the mechanisms involved.

**Rationale:** The ability of membranes to selectively remove and/or separate metal ions would allow their application in areas such as catalyst recycling, water purification, electroplating, and possibly even ion sensing. In order to develop membranes with these properties, membrane construction and the mechanism of ion transport must be understood.

**Approach:** The initial portion of this study has focused on fixed site carrier membranes—solid polymeric membranes with crown ether functionalities polymerized into the polymer backbone. Crown ethers were chosen for the initial study because there have been extensive studies done on these compounds, and their metal ion complexing ability is well known and published in the literature. The crown employed in the initial work is 4'-vinylbenzo-18-crown-6 which has a cavity size that accommodates the potassium ion. A co-monomer, di(ethylene glycol) ethyl ether acrylate (DEGEEA) was chosen because of its molecular similarity to the crown and because of its lesser toxicity relative to other species of this type. The choice of a co-monomer allows manipulation of the functionalization (mole percent of the crown ether in the monomer mixture) and construction of membranes with a wide range of compositions. A small amount of cross linking agent (1-5 mole percent hexamethylene diacrylate—HMDA) has been employed in the monomer mixture so that a lightly cross linked polymer product results. A microporous polyethylene support provides mechanical integrity and the monomer system is photopolymerized within this matrix. The solid membranes are mounted in a transport cell and experiments with varying aqueous solutions are conducted. The ion transport of potassium, sodium, rubidium, and cesium through membranes of varying composition has been investigated to date with some single component and some multi-component experiments. The effect of the anion which accompanies potassium has been investigated on a preliminary basis. When potassium feed solutions of comparable concentration are tested with each of the perchlorate, nitrate, and triflate salts, an unexpected result was obtained. The potassium ion flux was greater when nitrate was the counter ion than that with either potassium perchlorate or potassium triflate. This trend would not be expected when the free energy of hydration of the respective anions is considered. Ion flux is affected by both the feed solution concentration and the level of functionalization in the membrane. The potassium ion flux has a squared dependence on the feed solution concentration. The flux has a more complex dependence on the level of functionalization. It starts with no flux at low levels of functionalization and exhibits an increasing trend until levels of approximately 40 mole percent of the crown are reached, whereupon the flux displays a saturation-like behavior. An assessment of membrane material properties with solid state Nuclear Magnetic Resonance and Atomic Force Microscopy is planned. The water uptake and swelling properties of the membranes are currently being investigated.

**Status:** This project commenced in January of 1996 and is a part of the NSF/IU/CRC Center for Separations Using Thin Films at the University of Colorado. Research is expected to be complete between late 1997 and mid 1998.

# DETOXIFICATION OF METAL-CONTAMINATED WATERS USING SHELLFISH WASTES

**Helen E. A. Tudor**
Department of Chemical Engineering, Materials Science and Mining Engineering
Columbia University

**Goal:** To develop a rapid, simple, and cost-effective process for the removal of heavy metals from polluted waters using recovered shellfish industry wastes.

**Rationale:** This research addresses two pollution problems arising from industry: water contamination by heavy metals, and solid wastes currently characterized as pollutants generated by seafood processors. The process being developed would recover solid wastes for utilization as secondary raw materials. Regarding the heavy metals, the aim is to develop an efficient and versatile removal process which can be practiced on a small or large scale and used alone or in concert with other existing technologies. It would also reduce the amount of sludge in comparison with presently used methods while maintaining or improving upon current discharge requirements.

**Approach:** Six steps are involved: (1) Prepare crustacean (lobster, crab, shrimp) and mollusk (clam, mussel, oyster) shells in appropriate size ranges; (2) select heavy metals (lead, cadmium, etc.) representative of effluents generated by industries such as mining, electroplating, battery manufacturing, etc.; (3) measure the kinetics of metal uptake from solution and sorption isotherms—several types of aqueous solutions, including multi-metal solutions, will be tested to determine the effect of different anions, ionic strength, and salinity; methods for recycling the reacted shell will also be explored via elution studies at different pHs; (4) study the structure of the reacted shell to help elucidate the mechanism of the sorption process; (5) develop mechanistic predictive models of the sorption process; and (6) develop a hydrometallurgical process for extracting metals from ores based on the current research.

**Status:** Initial tests with lead, cadmium, and copper have been carried out on several types of shell. All displayed very fast initial kinetics (the entire process being completed within the first several minutes), followed by much slower final kinetics, especially in the higher concentrations. Detailed experiments with lead in the range 10-30,000 ppm over a time scale range of 5 minutes to 14 days confirmed these findings. Preliminary analyses of the shell substance showed considerable structural changes following exposure. Of special interest are the results at the highest concentrations (20,000 and 30,000 ppm) which showed that shell can abstract virtually the entire mass of metal from solution, equal to approximately 150% of the initial weight of the shell. This magnitude of uptake is considerably greater than the amount expected due to either an ion-exchange or chelation mechanism. In contrast, *Chitosan,* a widely researched and advocated material for water purification, used here for purposes of comparison, displayed slower kinetics and considerably lower final sorption capacity. Further detailed experimentation is planned in order to develop models describing the process and methods for recovery of the metals and recycling of the shellfish reactants. Estimated time of completion is 1998-99.

# IMPLICATIONS OF NICOTINE INTERACTIONS WITH INDOOR SURFACES ON ITS USE AS A MARKER FOR ENVIRONMENTAL TOBACCO SMOKE

**Michael D. Van Loy**
Department of Civil and Environmental Engineering
University of California at Berkeley

**Goal:** To elucidate the mechanisms by which semivolatile organic compounds (SVOCs) interact with indoor surfaces, and to develop models to accurately describe these processes, using nicotine as a marker for environmental tobacco smoke (ETS) exposures in indoor air.

**Rationale:** Nicotine has been widely used as a marker for ETS because its only significant source in indoor air is tobacco product combustion, it is easily detected, and it is emitted from different types of cigarettes at similar rates. A strong correlation between nicotine concentrations and those of respirable suspended particles and other ETS constituents has been reported in indoor environments where smoking occurs. However, other researchers have questioned the suitability of nicotine as a marker for ETS based on laboratory chamber study observations of differences between its dynamic behavior and that of other ETS constituents. Currently, the debate over nicotine's utility as an ETS marker remains unresolved. Elucidation of the factors affecting nicotine concentrations in indoor environments is necessary to improve the basis for using nicotine to assess ETS exposures. This research also has potential applications in predicting exposures to other SVOCs or in estimating the importance of gas particle partitioning to the fate and persistence of these compounds in indoor air.

**Approach:** This study employs recently developed models describing the interactions of gas-phase semivolatile organic compounds with indoor surfaces to examine the effects of sorption on the suitability of nicotine as an ETS marker. Using data from studies of nicotine sorption on carpet, wallboard, and stainless steel, the dynamic behavior of nicotine in both real buildings and laboratory test chambers is modeled. In this manner, previously reported discrepancies in the behavior of nicotine in indoor air are resolved. The results indicate that nicotine may be a viable marker for ETS provided that the effects of adsorption and desorption are accounted for. In indoor environments where smoking occurs on a fairly regular basis for an extended period, the sorbed mass of nicotine becomes very large relative to the mass emitted by a single cigarette, and re-emission from indoor surfaces dominates the gas-phase concentration. Where smoking occurs less regularly, the rate of sorbed mass re-emission is not rapid enough to dominate direct emission.

**Status:** The final set of experiments for this research is being completed. Experimental work is planned for completion by the beginning of this summer. Barring any significant setbacks, dissertation write-up should be complete by the end of Fall, 1997.

# DETERMINING RESPONSES OF TROPICAL RAIN FOREST CANOPY PHOTOSYNTHESIS TO CHANGES IN CLOUD COVER: A BOTTOM-UP APPROACH

**Lee A. Vierling**
Department of Environmental, Population, and Organismic Biology
University of Colorado, Boulder

**Goal:** To determine how variations in cloud cover impact tropical rain forest canopy light and temperature environments, and how these changes may influence photosynthesis at the canopy scale.

**Rationale:** Tropical rain forest ecosystems commonly experience extended periods of cloud cover. Because atmospheric conditions affect the diffuse fraction (the intensity and source direction) of the photosynthetically active radiation (PAR) field, variations in cloud cover are likely to significantly impact canopy PAR penetration and canopy $CO_2$ exchange with the atmosphere. Previous investigations above forested ecosystems have reached contrasting conclusions regarding the effect of cloudiness on net ecosystem $CO_2$ exchange, and a comprehensive process-level data set has not been available previously to assess this relation in a tropical rain forest. By addressing this issue, this research aims to elucidate the dynamic role that tropical rain forest ecosystems play in the global carbon cycle.

**Approach:** This study integrates three main sources of information: field studies, greenhouse experiments, and simulation modeling. Field measurements were made during November and December, 1996 within a monospecific *(Gilbertiodendron dewrevrei)* primary rain forest located in the Republic of Congo, Central Africa. In the field, cloud cover was quantified by continuously measuring the sky diffuse fraction from atop a tower extending above the canopy. At the same time, a suite of physical and ecophysiological measurements was gathered at various depths throughout the canopy. These measurements included detailed quantification of PAR, leaf temperature, leaf area index, and leaf angle distribution. In addition, *in situ* measurements of leaf photosynthesis with varying light, temperature, and intercellular $CO_2$ were made using a portable photosynthesis instrument. To supplement these field data, measurements of photosynthetic activity from *Gilbertiodendron* saplings will be made in the greenhouse. A system will be developed to expose the saplings to light and temperature environments similar to those measured in the field. A critical greenhouse measurement will be to quantify photosynthetic induction, a phenomenon that controls the carbon economy of a leaf during lightflecks. The modeling component of this study will synthesize field and greenhouse measurements to arrive at a multiple-layer representation of canopy photosynthesis under various cloud conditions. This model will hopefully be validated in the future with tower eddy covariance measurements of $CO_2$ flux.

**Status:** Field data from last winter are currently being analyzed. Greenhouse measurements are planned for Summer 1997, and a computer model will be developed in Fall 1997. Research should be complete by Summer 1998.

# CONTROLLING CHAOTIC BEHAVIOR IN LEAN COMBUSTION SYSTEMS

**Robert M. Wagner**
Department of Mechanical and Aerospace Engineering and Engineering Mechanics
University of Missouri-Rolla

**Goal:** To apply chaos theory in developing control systems for internal combustion engines for effective operation near the lean combustion limit.

**Rationale:** Cycle-to-cycle variations in internal combustion engines have long been recognized as a limiting factor in engine performance, fuel efficiency, and emissions. In particular, cycle-to-cycle variations are a major factor during lean operation. Lean operation is desired to reduce nitrogen oxides and hydrocarbon emissions as well as to improve fuel efficiency. Traditional control techniques are not capable of meeting increasingly aggressive emissions and fuel efficiency goals. Hence, new approaches must be investigated in an effort to solve these problems. The successful completion of this investigation will result in a more effective utilization of the lean combustion limit and, correspondingly, an increase in fuel efficiency and a reduction in emissions.

**Approach:** This research aims to accomplish the following: (1) identify regions of deterministic chaos in a realistic spark-ignition engine, (2) investigate the dependence of combustion stability on engine operating conditions, and (3) develop control algorithms based on chaos theory to reduce cycle-to-cycle variations near the lean combustion limit. The development of chaos-based control and analysis algorithms for an internal combustion engine will entail five steps: (1) development of a realistic engine model for computer simulations, (2) investigation of the dependence of combustion stability on the engine model operating conditions, (3) development of an algorithm to control chaotic behavior in the engine model, (4) investigation of the dependence of combustion stability on the operating conditions of an actual engine, and (5) development of an algorithm to control chaotic behavior in an actual engine.

**Status:** An engine model developed at Oak Ridge National Laboratory (ORNL) is being used for computer simulations in this investigation. The ORNL model is in the process of being modified to include the effects of flame temperature, extinction, and ignition. Experiments are also being performed to gain a better understanding of the impact of fuel and air mixing on combustion dynamics. The experiments focus on the transition from stable to unstable combustion as equivalence ratio is decreased until the lean limit is detected based on cylinder pressure. Results of preliminary experiments performed on a single-cylinder spark ignition engine with no fuel and air mixing enhancement indicate engine behavior near the lean limit is deterministic and chaotic in nature. The next phase of experiments will be performed with a shrouded intake valve to enhance the fuel and air mixing process. The results of the two groups of experiments will provide insight into the impact of the fuel and air mixing process on the nonlinear combustion dynamics and the mechanisms dictating the location of the lean limit. A better understanding of these relationships is necessary in designing an effective control system.

# REDUCTIVE DECHLORINATION OF MODEL CHLOROPHENOLS IN ESTUARINE SEDIMENTS: ROLE OF SULFATE REDUCTION

**Kimberly A. Warner**
Marine, Estuarine, and Environmental Science Program
University of Maryland

**Goal:** To understand how chlorinated aromatic hydrocarbons (CAHs) fit into the complex interactions in natural estuarine anaerobic food webs dominated by sulfate reducing bacteria (SRB).

**Rationale:** The potential for reductive dechlorination (RD) in marine sediments has received little scrutiny due to the observed inhibition of this microbial process by sulfate in freshwater systems. This research will attempt to identify important controls on toxic CAH degradation in coastal environments, provide useful information for bioremediation strategies, and improve understanding of estuarine sediment microbial ecology.

**Approach:** Specific questions to be addressed are: Is RD an important fate for CAH pollutants in sulfate-rich coastal environments? and What is the role of SRB and other physiological bacterial groups and environmental constraints on this process? This research uses a combination of field and laboratory studies to assess the ability of natural microbial assemblages in sediments to dechlorinate reductively the model compound, 2,4-dichlorophenol (DCP), along the salinity gradient of Chesapeake Bay. Whole sediment from distinct depth horizons at three well-characterized benthic sites were dosed with DCP, and short term RD activity was assessed in relation to net hydrogen production, down-core rates of sulfate reduction and *in situ* levels of sulfate, sulfide, methane, and acetate, as well as to seasonal deposition of carbon at a mid-bay (MB) site. Laboratory experiments examined RD activity in anaerobic MB slurries both in the absence and presence of sulfate, added electron donors, and metabolic inhibitors of SRB and methanogenic bacteria, while monitoring relevant biogenic gases and solutes. Using similar experimental protocols, sulfate-rich, non-reducing sediments of the lower-bay (LB) were examined for RD and degradation potential of DCP under both anoxic and oxic conditions.

**Status:** The bulk of the field and laboratory investigations have been completed and data are being analyzed. Preliminary results prompted some changes in direction and opened up further intriguing questions which may or may not be addressed within the context of this dissertation. Field investigations revealed the short-term onset of RD activity at all three primary sampling sites regardless of apparent sulfate concentrations. In surficial MB sediment, two rates of RD can be described: a slow steady rate in the presence of sulfate and a very rapid rate upon the depletion of sulfate and concomitant increases in electron donor (hydrogen) levels. SRB participate in the RD process at the two sites tested, though the behavior and populations of SRB appear to vary with depth and site. At MB, SRB play a critical role in RD whether or not sulfate is present and appear to switch metabolic pathways when sulfate is absent, leading to rapid RD. At LB, RD proceeds readily in the presence of sulfate and without a lag. Remaining research includes: a) determination of background halophenols in these sediments which may have adapted the microbial flora to readily degrade these compounds; b) assessment of the role of SRB at MB as agents of RD or suppliers of electron donors for the reaction under fermentative growth mode; and c) follow-up on apparent site- and depth-specific inhibition of sulfate reduction in the presence of DCP. Research is planned for completion by the end of this year or early in 1998.

# NEW STATISTICAL METHODS IN ENVIRONMENTAL EPIDEMIOLOGY: APPLICATION OF LATTICE DIAGRAMS

**Thomas F. Webster**
Department of Environmental Health
Boston University School of Public Health

**Goal:** To evaluate the usefulness of the lattice diagram in addressing some common issues in environmental epidemiology: crude exposure information, categorization, and small numbers of exposed cases.

**Rationale:** Environmental epidemiologists often study the relationship between exposure and disease risk by categorizing exposure (low, medium, or high). How many categories to use and where to place the boundaries (cut-points) must be decided, and such decisions affect the precision of results and may cause bias. Another kind of misclassification is caused by the crude nature of many measures of exposure (distance of residence from a hazardous waste site). Although many investigators believe that nondifferential misclassification biases results towards the null, this is not true in general. Counter examples include multiple exposure categories for which the direction of bias is not completely understood and ecological (group-level) analyses.

**Approach:** Suppose that for each case and control in an epidemiological study there is an ordered measure of exposure (concentration of a single pollutant). In a lattice diagram, the number of exposed cases is plotted on the Y-axis and the number of exposed controls is plotted on the X-axis. Each point on the lattice represents a possible study outcome. If, starting at zero, the level defined as exposed is gradually increased, a non-decreasing curve is produced on the diagram. This pathway is related to receiver-operating characteristic (ROC) curves used in medical diagnostics. Some applications are: (1) Choice of category boundaries: The pathway summarizes all possible 2x2 tables generated from the data set by all possible cut-points. A line segment joining any two points on the pathway defines an exposure category with a slope equal to the case-control ratio for that category, a number proportional to risk. Approximating the pathway with a series of line segments constructs a descriptive categorization. (2) Hypothesis testing: The lattice diagram is useful for suggesting and comparing statistics. An underlying model of independent binomials as well as Fisher exact, chi-square, and the non-parametric Kolmogorov-Smirnov and Wilcoxon statistics can be pictured. The latter two can test the deviation of the entire pathway from the null, represented by the diagonal of the diagram. Expected values of the statistics can be computed for different exposure distributions and exposure-response relationships. Power can be investigated theoretically and via simulations. (3) Nondifferential exposure misclassification: A lattice diagram or the closely related risk diagram can be used to study misclassification of cases and controls categorized by exposure. In a risk diagram, the risk (or case-control ratio) for each category is plotted along a separate axis. The results of a study are given by a vector. Non-differential exposure misclassification can be thought of as a linear transformation of the risk vector by a row stochastic matrix. Such matrices incorporate information on the relative size of exposure categories (as well as sensitivity and specificity). The null vector (the vector of equal case-control ratios) is a positive eigenvector with eigenvalue equal to one.

**Status:** Significant progress studying categorization and misclassification with the aid of the lattice diagram has been made this year. Next year, more attention will be focused on using the lattice diagram to compare statistics of pathways. The use of non-parametric regression is being examined as another approach to these problems. Research is scheduled for completion in about one more year.

# WHEN YOU CAN ONLY DO SO MUCH: BUDGET CONSTRAINTS AND RESPONSES TO CONTINGENT VALUATION SURVEYS

**Ned Welch**
Department of Social and Decision Sciences
Carnegie Mellon University

**Goal:** The purpose of this work is to understand how people reason about their own finite resources when answering questions posed by policy researchers about the value of environmental resources.

**Rationale:** One frequently used methodology for eliciting public values, the Contingent Valuation Method (CVM), infers the social value of an environmental good by asking a representative sample of citizens to evaluate the amenity and state their maximum willingness to pay (WTP) for it. Budget constraints are an important normative consideration which has received only scant attention in the literature on CV. A normatively correct analysis of WTP must incorporate the evaluator's "wealth." This wealth increases with the number of public goods the evaluator currently "owns" and decreases with the number of goods that are currently being evaluated or that may soon require intervention. However, CVM studies typically do not provide the subject with information about other goods being evaluated.

**Approach:** The first set of studies examined how budget constraints affect people's evaluations of a proposed policy change. Study 1 looked at within-subject effects of added information about the budget problem. First, respondents completed an CVM-like evaluation task. They then were offered opportunities to reflect on additional information about the target good and about the evaluation methodology (including budget constraints) that they might consider relevant to their valuation. The variables of interest included changes to stated WTP in response to the additional information and changes in a variety of attitudes about CV. The research asked whether respondents adjusted their WTP when budgetary issues are presented, and, if WTPs are not easily changed, as some research suggests, will other judgments relevant to the valuation procedure be more sensitive?

Study 1 respondents infrequently adjusted their WTP, although they did change other judgments (*e.g.*, their endorsement of CV as a policy-making tool and their preference for abstaining from the valuation exercise). To address the possibility that our elicitation procedures had "anchored" respondents on their initial stated WTP, we replicated the experiment in a between-subject design. In Study 2, half the subjects replicated the conditions of Study 1, evaluating the target good, then evaluating it again after receiving information explaining the CVM context. The remaining subjects read the context information first, then performed the valuation task.

**Status:** The results of these studies suggest that respondents care about budget constraints but have a difficult time incorporating these concerns into WTP judgments. A new study, currently in the design phases, examines the behavior of a (simulated) social policy system in which priorities are guided by WTP judgments. When evaluators have imperfect information about what other goods are being evaluated and the impact of previous valuation exercises on future taxes, the system may manifest interesting dynamic properties

# PREDICTING ELECTRON TRANSFER AND TAILORING CHEMICAL MICROENVIRONMENTS

**Mona Wells**
Department of Chemistry
Texas A&M University

**Goal**: To explore the role of novel supramolecular architectures as microenvironments and to elucidate the mechanism of electron transfer (ET) operative at the surface of colloidal semiconductor catalysts.

**Rationale**: Electron transfer processes are critical to natural phenomena such as photosynthesis and enzyme catalysis. Mimicry of these natural processes holds the key to development of more efficient technology for use in photocatalysis, energy storage, and energy transduction. To ultimately harness the potential of ET, the optimum chemical environment and how to create that environment with synthetic techniques that are relatively facile needs to be determined.

**Approach**: Three-dimensional supramolecular architectures are large enough to function as hosts to smaller guest species and thus function as chemical microenvironments. In this study, Merrifield protocols for protein synthesis and D. Tomalia's divergent growth technique for dendrimer synthesis were adapted to surfaces to create three new types of supramolecular architectures, namely surface-grafted dendrimers, surface-confined dendrons, and surface-grafted hyperbranched polymers. These constructs were initially attached to thiol-based self-assembled monolayers on gold-coated silicon for characterization but are also amenable to attachment on more mundane surfaces such as silica or $TiO_2$. Two series of surface acoustic wave sensors with the grafted-dendrimer and hyperbranched polymer architectures, respectively, were exposed to a suite of vapor-phase dosants, and the surfaces exhibited interesting molecular recognition capabilities. These architectures can be synthesized in thicknesses varying from about 10 nm to greater than 1000 nm, and with an almost unlimited choice of chemical functionality, illustrating how microenvironments with tailor-made properties may be constructed. The colloidal semiconductor $TiO_2$ is an excellent surface platform for the type of constructs described here and is a readily available, inexpensive, relatively inert material with photocatalytic properties in a wavelength range encompassing that of ambient sunlight.

Photoinduced electron transfer between $TiO_2$ and a second species is the first step in catalytic photodegradation, and empirical evidence indicates that the effect of chemical environment on this process is crucial. In terms of catalyst design however, predictive capability is desirable. Theoretically predicting the effect of microenvironment on photoinduced ET involves development of a model, and the model chosen here is based upon scattering theory. Before applying scattering theory, *ab initio* techniques are used to calculate a set of orbitals for the molecular target. The energies of these orbitals are then compared to experimental values of ionization potentials. Once a set of orbitals is obtained with reasonable orbital energies, scattering theory is used to determine the mechanism of electron entrapment and decay of the excited state. Thus far, comparison of results from this theoretical formalism to experiment have verified its utility. Insight into the role of microenvironment in photoinduced ET is obtained by comparing photoionization of a lone target to photoionization of a target in the presence of secondary chemical species.

**Status**: The molecular architecture portion of the project is complete and has resulted in three publications and two patents pending. Preliminary scattering calculations are being performed on simple targets, and the mathematical and computational aspects of extending the existing computer codes to include effects of chemical microenvironment are being developed. It is hoped this research will be completed sometime this century.

# RELATING RELEASE AND BIODEGRADATION KINETICS OF AGED HYDROCARBONS IN SOILS

**Derek G. Williamson**
Civil Engineering
The University of Texas at Austin

**Goal:** To uncouple mass transfer and biodegradation kinetics of aged hydrocarbons from field soil samples in order to estimate the relative contribution of each process to fate of hydrocarbons in soil systems.

**Rationale:** There is relatively little readily available data describing the kinetics of release of aged hydrocarbons from soils, and even less examining the role of release on biodegradation of aged complex hydrocarbon mixtures. Such information may be critical to site evaluations of risk-based cleanups and intrinsic biodegradation initiatives.

**Approach:** A laboratory protocol is used to estimate the Rate of Release (ROR) of hydrocarbons from aged soils over extended periods of time (months). Soils collected from manufactured gas plant (MOP) sites have been used in this protocol, with 2- and 3-ring PAHs being the compounds of concern in this research. The ROR protocol is a sacrificial technique in which a series of test tube reactors are loaded with soil, XAD2 resin, and a solution containing $HgCl_2$ and $CaCl_2$, tumbled end-over-end, and removed for analysis at various time intervals. The XAD2 resin, extracted with solvent, rapidly removes hydrophobic organics from the aqueous phase, maintaining nearly zero concentration of the desorbed compounds in the aqueous phase. This protocol allows a much larger portion of the hydrocarbons associated with the soil to be released than is possible with techniques that measure kinetics as an approach to equilibrium. The collected data are analyzed with an empirical two-site model consisting of two first order rate constants ($k_1$ and $k_2$) describing fast and slow releases, respectively, and F, the fraction that is rapidly released.

The biodegradation experiments are conducted in a manner analogous to the ROR experiments. Again, a sacrificial technique is used with a series of batch reactors tumbled end-over-end. The reactors contain a slurry of soil and an oxygen saturated nutrient solution. The biodegradation experiments were designed to alleviate any limitations (other than mass transfer) to biodegradation. This allows the impact of mass transfer on the biodegradation process to be isolated from environmental limitations. Sacrificial sampling includes extraction of the aqueous phase and the soil phase. A coupled process model consisting of a mass balance and measured ROR kinetics is applied to the ROR and biodegradation data to estimate a first order aqueous phase biodegradation coefficient ($k_{bio}$).

**Status:** Several release and biodegradation experiments have been performed. Preliminary data are available from some experiments and additional data are being processed. A spreadsheet version of the coupled process model has been developed and applied to some data; however, a more robust modeling tool will need to be developed. The bulk of the initial experimental work should be completed by mid-summer 1997. Some additional lab work may be indicated as collected data are further analyzed.

# CONTROL OF DBPs IN DRINKING WATER USING THE OZONATION/FBT PROCESS

**Alex A. Yavich, P.E.**
Department of Civil and Environmental Engineering
Michigan State University

**Goal**: To establish the technical and economical merits of the ozonation/biological fluidized bed treatment (FBT) process for disinfection by-product (DBP) control in drinking water.

**Rationale**: A significant portion of the water treatment industry will face technical and financial hurdles in meeting the DBP requirements of the SDWA (Safe Drinking Water Act) reauthorization. In addition, in compliance with SDWA amendments, the US EPA will be required to identify technologies that are technically and economically feasible for small systems serving up to 10,000 people. If technical and economical merits are established, the ozonation/FBT process will be a very attractive alternative to both conventional coagulation and ozonation/biofiltration processes, especially for small water treatment utilities and larger utilities where space for retrofitting or expansion is limited.

**Approach**: One of the distinctive features of the proposed process is the use of a fluidized bed reactor (FBR) in place of biofilters. This makes the ozonation/FBT process advantageous over conventional combined ozonation/biofiltration processes for the following reasons:

(1) Clogging is virtually impossible in FBRs and, therefore, no additional pretreatment (e.g., flocculation/sedimentation) to remove turbidity is required.
(2) The removal efficiency for biodegradable organic matter is greater with FBR treatment than with biofiltration because the specific surface area and the attached biomass concentration are greater in the FBR.
(3) The process can be operated in a cyclic mode, in which water alternately passes through ozonation and biodegradation steps; in this way, ozonation is used to degrade only those compounds that are not biodegradable, thus reducing ozone consumption and related capital, operating, and maintenance costs.
(4) The performance of the ozonation/FBT system can be optimized for different sources of water supplies. The system is also expected to meet future effluent standards through process parameter optimization without expansion or modification.

Bench-scale and small pilot-scale studies are being conducted with Huron River water, a source water for the Ann Arbor Water Treatment Plant. This plant uses lime softening, flocculation/sedimentation, ozonation, and GAC filtration. The pilot ozonation/FBT system includes an ozone contactor, a retention tank, and a FBR. No additional treatment is provided. The parameters that are monitored along the treatment train include pH, alkalinity, turbidity, TOC (total organic carbon), THMFP (Trihalomethane Formation Potential), chlorine demand, UV254, humic substance concentration, and aldehydes.

**Status**: Research has shown that the process is able to remove up to 60% TOC, up to 80% THMs and chlorine demand, and up to 90% of color, UV254, and turbidity. Upon completion of ongoing optimization studies, the ozonation/FBT process is expected to outperform the Ann Arbor process. For example, the study has already shown that recycling of a portion of the system effluent to the ozone contactor reduced ozone requirements by up to 50%. The project is expected to be completed in summer 1998.

# EXAMINATION OF STORMWATER TOXICITY USING THE MICROTOX BIOASSAY

**Cecelia Youngblood**
Department of Civil Engineering
University of Illinois at Urbana-Champaign

**Goal:** To measure the toxicity of low concentrations of binary and complex mixtures of contaminants using the Microtox bioassay, and determine if pollutant mixtures at low concentrations are responsible for inducing the high levels of hormesis (stimulation of activity when organisms are exposed to low levels of toxics) previously measured in stormwater samples

**Rationale:** Stormwater runoff contains a complex mixture of contaminants including heavy metals, oils and greases, and organic chemicals. Although individual profiles of toxicity are available for many contaminants in stormwater, few studies have been conducted which examine the toxicity of mixtures of substances. Since toxicants rarely occur individually in nature, it is critical to determine how organisms are affected by various combinations of pollutants. Stormwater runoff analyzed using the Microtox assay often results in high levels of light stimulation (up to 60%). A previous study using the Microtox assay demonstrated that stimulation was produced when *Vibrio fisheri* were exposed to very low concentrations of individual metal or organic toxicants. This stimulation is considered a *hormesis* effect, a general term for stimulation of activity when organisms are exposed to low levels of contaminants. This research extends the previous hormesis study by examining the effects of binary and complex mixtures of toxicants using low, environmentally realistic concentrations of contaminants in order to examine stormwater effects.

**Approach:** Experimentation will be conducted in two phases: Binary Mixture Experiments and Stormwater Simulation Experiments

*Phase 1:* The Microtox screening test will be used to test pairs of metals, organic chemicals, and metal-organic combinations. A 5x5 matrix of concentrations will be used for each binary combination, resulting in 25 treatments for each pair of toxicants. Each 5x5 matrix will be replicated three times using a different lot of reagent for each replication to account for biological variability. All Microtox testing will be performed according to standard procedures recommended by Azure Corporation. Because the toxicity of mixtures may be affected by temperature, each experiment will be replicated three times at both 15°C and 23°C. Five metals and five organic chemicals will be selected for study from the following list: cadmium, chromium, lead, nickel, zinc, phenol, sodium laurel sulfate, 1-octanol, benzene, acetone, ethylene glycol, atrazine, acetaldehyde, diethylene glycol. The substances chosen represent common environmental contaminants and a range of functional groups. Appropriate hormesis-inducing concentrations of each toxicant will be determined prior to the matrix experiments through the construction of individual dose response curves.

*Phase 2:* After the binary combination experiments have been completed, various combinations of toxicants (mixtures containing >2 toxicants) will be created. Previous Microtox screening tests performed on stormwater samples resulted in stimulation as high as 60%; however, no individual toxicant concentration have ever stimulated such a large percent effect. Using the data collected in Phase 1, combinations of chemical groups will be tested to produce high levels of stimulation. Due to the enormous number of combinations that a factorial design would require, only selected mixtures will be analyzed. Some selected mixtures will have toxicant concentrations based on actual concentrations measured in collected stormwater samples.

**Status:** Phase I and Binary mixture experiments are in progress, and dose-response curves for individual toxicants are complete. Expected project completion date is December 1997.

# THE EFFECT OF NITROGEN DEPOSITION ON THE GROWTH AND COLONIZATION OF MYCORRHIZAL PLANTS

**Lidia Ceballos Yoshida**
Department of Botany and Plant Sciences
University of California, Riverside

**Goal:** To determine the effects of two forms of nitrogen, ammonium ($NH_4$) and nitrate ($NO_3$), on the growth and mycorrhizal response of a coastal sage scrub species, *Artemisia californica* Less., and an exotic annual grass, *Bromus madritensis* L.

**Rationale:** Anthropogenic nitrogen deposition is causing known vegetation changes to plant communities but less is known about its effect on mycorrhizae. It is possible that nitrogen deposition may be influencing the decline of coastal sage scrub in favor of exotic grasses by affecting the functioning of the mycorrhizal fungi colonizing the roots.

**Approach:** Seeds of *Artemisia* and *Bromus* were germinated in pots in two types of soil inoculated with native soil containing mycorrhizal spores. Two soil types were used; one with high (30 ppm) and one with low (7 ppm) phosphorus. All pots were fertilized with all mineral nutrients except phosphorus and nitrogen and received a nitrogen treatment (50 ppm $NO_3$ or $NH_4$) or a control treatment prior to sowing. Soil samples will be taken at the beginning, middle, and end of the experiment and analyzed for nitrogen content. The roots and shoots will be harvested, dried, and weighed for biomass determination eight weeks after germination. The shoots will be ground and analyzed for percent carbon and nitrogen. The roots will be stained with trypan blue and percent root infection will be determined microscopically.

**Status:** Preliminary experiments are completed to select appropriate soil types, inoculum type, and timing of plant growth and mycorrhizal infection. The main experiment was begun during the first week of March 1997. The plants are expected to be harvested early May 1997. Completion of this experiment is expected by the end of May 1997. A preliminary greenhouse study utilizing $^{15}N$ to examine uptake of nitrogen by mycorrhizal *Artemisia* and *Bromus* plants in competition is in the planning stages. Additionally, field experiments are planned to replicate the above studies in a coastal sage environment. The $^{15}N$ and field studies should be completed by fall 1998.

# THE ROLE OF TIDAL CURRENTS IN DETERMINING THE TIMING AND LOCATION OF ENERGY TRANSFER IN MARINE FOOD WEBS

**Jeannette E. Zamon**
Department of Ecology and Evolutionary Biology
University of California, Irvine

**Goal:** To define the physical and biological mechanisms responsible for creating and maintaining elevated levels of energy transfer through a food web associated with a tidal channel in Puget Sound, Washington, and to develop a conceptual model of energy transfer that will allow prediction of how three types of human disturbance (fishery activity, acute pollution, and chronic pollution) are likely to effect this food web.

**Rationale:** This research project has both specific, applied importance, and general ecological importance. First, the location and timing of a gillnet fishery near the study site has the potential to impact seabirds and marine mammals by entangling these animals in the nets. Determination of the temporal and spatial feeding patterns of top predators will help resource managers design net deployments which minimize undesirable impact. Second, the study site is situated near several shipping lanes leading to an oil refinery. Effective oil spill response strategies need to be based on a knowledge of how tidal changes in surface currents and in habitat use by predators might influence the area and species impacted by spills. Third, predicting the fate of contaminants requires knowledge of the physical and biological pathways linking species within the food web. Lastly, several studies have observed that organisms aggregate to feed in tidal channels of tropical, temperate, and polar seas, but little is known about why this occurs in marine food webs. This is an area of marine ecology that needs to be investigated.

**Approach:** The research will attempt to determine spatial and temporal patterns in consumer distributions and feeding activity and to establish which of several potential mechanisms generates these patterns. Techniques from behavioral ecology and biological oceanography are being combined to systematically sample the distribution, abundance, and feeding activity of tertiary consumers (seabirds), secondary consumers (schooling fishes), and primary consumers (zooplankton) in relation to changes in the tidal currents which enter the pass. Sampling periods are determined from tidal current prediction tables. Sampling is spatially explicit because the study site has been divided into spatial sectors, and a positioning device is used to determine the latitude and longitude of seabird feeding aggregations, fish schools, zooplankton, and tidal currents.

Observational sampling is used to quantify the feeding activity of the seabirds. A 120 kHz, single-beam echo sounder is used on a boat to map fish distributions. Bird observations are made from a land-based site. A dip net is used to identify fish species and fish diets, and a series of plankton nets from the boat are used to sample tidal changes in zooplankton distributions. Finally, a quantitative temperature-salinity probe and surface drifters are used to determine the origin and destination of water and zooplankton that flood into and ebb out of the study site.

**Status:** Pilot studies in 1995 and 1996 determined that feeding activity of top predators is contingent upon the presence of schooling fish. When fish are present, feeding activity is highest during the first half of flooding tides. This peak in feeding activity appears to be correlated with increases in fish abundance in the first 25 m of the water column and with increases in the near-surface abundance of zooplankton. Evidence from 1996 temperature probe deployments suggests zooplankton originate in surface waters exchanged during the tidal cycle. During 1997, the origin of increased zooplankton abundances with respect to the movement of specific water masses and areas of peak seabird activity and fish abundance will be investigated. Data collection, analysis, and publication of this research is expected to be completed in mid-to late 1998.

# SELECTIVE LOGGING EFFECTS ON BOTTOMLAND HABITAT QUALITY AS DETERMINANTS OF HABITAT OCCUPANCY BY ACADIAN FLYCATCHERS

**Gary D. Zenitsky**
Department of Biology
University of Memphis

**Goal:** To determine whether selective logging alters habitat quality enough to influence the distribution of breeding birds among habitat patches in a bottomland hardwood forest, and to assess the potential of these patches to satisfy brood-rearing demands and serve as source or sink habitats, using density-dependent habitat selection and central-place foraging theories.

**Rationale:** The Mississippi Alluvial Plain (MAP) is of critical importance to bird conservation. Besides supporting a diverse wintering and breeding bird community, floodplain forests in the MAP serve as stopover habitat in the most important migration corridor east of the Rocky Mountains. This productive ecosystem has been dramatically altered by levees, reservoirs, and land conversion to agriculture. The Acadian Flycatcher is a neotropical migrant exhibiting long-term population declines on the breeding grounds, and has been identified as a species of high management concern. The effects of various silvicultural prescriptions—no cuts, clear cuts, selection cuts—on the breeding biology of neotropical migratory landbirds in floodplain forests have not been studied. Results from this study will assist land managers in making informed decisions on how best to allow long-term, viable populations to exist.

**Approach:** Data are collected on 250 ha (hectare) uncut control and 250 ha select-cut plots. The ideal dominance model of density-dependent habitat selection requires age and biometrical data collected by mist-netting and color-banding adults. Song territories of males are mapped. Central place foraging data are collected from inside a 30 m radius, 15 m high cylindrical plot centered at a nest. The cylinder includes 8 equally spaced 30 m transects (spokes). All active nests are found and monitored, and during late brood-rearing, feeding territory volumes of adult females are mapped (tape-recorded) while nest provisioning by both adults is video-recorded. Afterwards, both left and right wing lengths of nestlings are recorded to calculate bilateral asymmetry as a measure of developmental stress. Arthropod biomass and percent territory openness are then sampled inside the cylindrical plots to represent habitat quality independent of the density and reproductive success of the habitat occupants. Foliage-dwelling arthropods are collected using a 4 m long sweep-net to collect 400 sweeps (0-6 m high) from live woody foliage and 50 sweeps from dead woody foliage along the 8 transects. Biomass is estimated from each specimen's length-width. Openness is estimated from vegetation (perch) height profiles, taken every 3 m on the 8 transects by recording a hit/miss for woody vegetation at 1 m height intervals from 0 m to the 15 m ceiling.

**Status:** Two of three 5-month field seasons with data from more than 50 nests have been completed. Data collection continues year-around because data from audio and video tapes and bottled insect samples must be extracted. Presently, a programmer is being consulted to develop software for extracting time budget data from audio tape. In 1998, field documentation of the location, age, and biometrics of birds settling on the 4 treatment plots will be made. Research is expected to be completed by August 1999.

# GEOPHYSICAL IMAGING OF SUBSURFACE CONTAMINANTS

**Jie Zhang**
Department of Earth, Atmospheric, and Planetary Sciences
Massachusetts Institute of Technology

**Goal:** To develop joint three-dimensional (3D) geophysical imaging methods for simultaneous reconstruction of multiple physical properties of subsurface contaminants from surface observations.

**Rationale:** It has long been recognized that specific survey objectives often cannot be met by applying only one geophysical method. A frequent problem is that insufficient contrasts of certain physical properties exist to detect the subsurface target of interest. In addition, geophysical interpretation suffers from ambiguity and limited resolution. The effort to develop joint imaging techniques, if successful, should lead to a major breakthrough in the area of contaminant characterization and monitoring.

**Approach:** Seismic and electrical properties of the subsurface contaminants are not directly associated with each other. If a large seismic velocity contrast occurs across a contaminant interface, certain changes in the corresponding electrical properties may also take place, but not necessarily. To seek solutions with maximum structural coherency between the two physical parameters in the earth, a joint seismic and electrical tomography method is developed that simultaneously reconstructs seismic velocities, electrical resistivities, and electrical chargeabilities with cross constraints on the normalized structural curvatures.

The joint approach combines a 3D nonlinear refraction travel time tomography method, 3D d.c. resistivity tomography method, and a 3D induced polarization tomography method. It includes a wave front ray-tracing method for rapid seismic travel time calculation and a transmission-network method for electrical potential calculation. A Conjugate Gradient (CG) approach is applied to solve an inverse problem that minimizes the seismic travel time and the electrical potential misfits while maximizing coherency of their normalized structure curvatures through the use of Tiknonov regularization.

**Status:** Research and development on the 3D electrical tomography and 3D seismic travel time tomography for characterizing contamination in the subsurface have been completed. A 3D induced polarization tomography approach will be developed. Combining three major 3D techniques, development of joint geophysical imaging methods is planned in order to reconstruct several physical properties of the contaminants simultaneously.

# *Office of Research and Development*
# *Contacts in*
# *Research Triangle Park, NC*

# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Center for Environmental Assessment
### Areas of Expertise

| | Telephone | Areas of Expertise |
|---|---|---|
| **NCEA-RTP Office** | | |
| *Office of the Director* | | |
| Lester D. Grant, Director | 919-541-4173 | Health effects of criteria air pollutants; heavy metals; global climate change; risk assessment |
| Michael A. Berry | 919-541-4172 | Environmental management; indoor environments; business and environment |
| Si Duk Lee | 919-541-4477 | Health risk assessment; international collaboration |
| William E. Wilson | 919-541-2551 | Aerosol (particulate matter) science; visibility; atmospheric chemistry; exposure assessment |
| *Environmental Media Assessment Group* | | |
| Larry J. Folinsbee, Chief | 919-541-2229 | Environmental and health effects of criteria air pollutants; cardio-respiratory physiology |
| Robert S. Chapman | 919-541-4492 | Epidemiology; respiratory physiology; Asian languages |
| Beverly M. Comfort | 919-541-4165 | Pesticides; indoor air pollution |
| Robert W. Elias | 919-541-4167 | Heavy metals; exposure modeling |
| William G. Ewald | 919-541-4164 | Toxicology; radiobiology |
| Jasper H.B. Garner | 919-541-4153 | Ecosystem and vegetation effects |
| D. Eric Hyatt | 919-541-0673 | Ecological assessment and policy decision theory |
| Dennis J. Kotchmar | 919-541-4158 | Epidemiology; respiratory effects; $NO_x$; PM health effects |
| Allan H. Marcus | 919-541-0636 | Statistics; epidemiology; pharmacokinetics |
| Joseph P. Pinto | 919-541-2183 | Atmospheric chemistry and climate change |
| James A. Raub | 919-541-4157 | Respiratory physiology/toxicology; carbon monoxide and ozone health effects |
| *Hazardous Pollutant Assessment Group* | | |
| J. Michael Davis, Acting Chief | 919-541-4162 | Developmental neurotoxicology; lead; manganese; oxyfuels; fuels and fuel additives; U-shaped dose response |
| Gary J. Foureman | 919-541-1183 | General metabolism; biological chemistry; general toxicology |
| Jeffrey S. Gift | 919-541-4828 | Health risk assessment; benchmark dose analysis; silica; glycol ethers; acrylates |
| Mark M. Greenberg | 919-541-4156 | Organic chemicals; physiologically based pharmacokinetics (PBPK) modeling; RfC methodology; isocyanates; asthma; benchmark dose analysis |

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## National Center for Environmental Assessment
## Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| Daniel J. Guth | 919-541-4930 | Pulmonary toxicology; inhalation risk assessment |
| Annie M. Jarabek | 919-541-4847 | Inhalation toxicology; risk assessment; dosimetry; PBPK modeling |
| Marsha L. Marsh | 919-541-1314 | Environmental health risk assessment; risk communication; urban toxics |

# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Exposure Research Laboratory
### Areas of Expertise

| | Telephone | Areas of Expertise |
|---|---|---|
| *Office of the Director* | | |
| Gary Foley, Director | 919-541-2106 | |
| Tom Clark, Deputy Director | 919-541-2109 | |
| *Associate Laboratory Directors* | | |
| Judy Graham | 919-541-0349 | Human exposure |
| Rick Linthurst | 919-541-4909 | Ecosystem exposure |
| *Assistant Laboratory Directors* | | |
| Tom Barnwell | 706-355-8441 | Research crossing traditional media (Athens, GA) boundaries |
| Bob Graves | 513-569-7619 | Water research (Cincinnati, OH) |
| Dale Pahl | 919-541-1851 | Research crossing traditional media (RTP, NC) boundaries |
| Gareth Pearson | 702-798-2101 | Hazardous waste research (Las Vegas, NV) |
| Chuck Steen | 706-355-8442 | Pesticides/toxic substances research (Athens, GA) |
| Jim Vickery | 919-541-2184 | Air research (RTP, NC) |
| **Air Exposure Research Division**<br>Mail Drop 56<br>Research Triangle Park, NC 27711 | | |
| Barry Martin, Acting Director | 919-541-4386 | Air monitoring research; air sampling and implementation design; acid deposition sampling; aerosol exposure research |
| *Exposure Assessment Branch* | | |
| Miriam Rodon-Naveira, Chief | 919-541-3075 | Human and ecosystem exposure study design, implementation, and interpretation |
| *Field Operations Branch* | | |
| Alan Hoffman, Acting Chief | 919-541-1929 | Air sampling and instrumentation design; aerosol exposure studies; air monitoring research |
| **Air Measurements Research Division**<br>Mail Drop 78A<br>Research Triangle Park, NC 27711 | | |
| Russ Wiener, Acting Director | 919-541-1404 | Air pollution aerosol technology; community and industrial hygiene |
| *Methods Branch* | | |
| Ross Highsmith, Chief | 919-541-7828 | Methods and protocols to measure toxic and hazardous air pollutants; ozone; volatile organic compounds; nitrogen oxides; automobile and stationary source emissions; personal/passive/ microenvironmental methods |

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## National Exposure Research Laboratory
## Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| *Quality Assurance Branch* | | |
| Andy Bond, Acting Chief | 919-541-4329 | Standards and systems to assure and control air monitoring data quality; Federal Reference Methods equivalency testing; National Performance Audit Program; Stationary Source Compliance Audit Program; data audits |
| **Characterization Research Division** | | |
| PO Box 93478 | | |
| Las Vegas, NV 89193-3478 | | |
| John Moore, Acting Director | 702-798-2525 | Systems engineering; systems analysis |
| *Analytical Sciences Branch* | | |
| Christian Daughton, Acting Chief | 702-798-2207 | Analytical chemistry; human exposure; earth sciences |
| *Monitoring Sciences Branch* | | |
| Bob Schonbrod, Acting Chief | 702-798-2229 | Ecosystem monitoring; landscape science; remote sensing |
| **Atmospheric Modeling Division** | | |
| Mail Drop 80 | | |
| Research Triangle Park, NC 27711 | | |
| Frank Schiermeier, Director | 919-541-4542 | Air quality modeling; air pollution meteorology |
| *Applied Modeling Branch* | | |
| Bill Petersen, Chief | 919-541-1376 | Human exposure modeling; fluid modeling simulations; criteria pollutant modeling; pesticides spray drift modeling; endocrine disruptor modeling |
| *Atmospheric Model Development Branch* | | |
| Jason Ching, Chief | 919-541-4801 | Regional transport and fate; ozone and particulate modeling; ecosystem exposure modeling; toxics transport/deposition modeling; boundary layer flux measurements |
| *Modeling Systems Analysis Branch* | | |
| Joan Novak, Chief | 919-541-4545 | High performance computing; scientific visualization; multimedia model development; pollutant emission methods |
| **Atmospheric Processes Research Division** | | |
| Mail Drop 77 | | |
| Research Triangle Park, NC 27711 | | |
| Larry Cupitt, Director | 919-541-2454 | Atmospheric chemistry |

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## National Exposure Research Laboratory
## Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| *Atmospheric Chemistry and Physics Branch* | | |
| Deborah Mangis, Chief | 919-541-3086 | Air pollution lifetimes and fate; physics of particulate pollutants; particle-bound pollutants chemistry and fate; chemical reactions and products; pollutant deposition |
| *Source Apportionment and Characterization Branch* | | |
| Roy Zweidinger, Acting Chief | 919-541-2324 | Automobile emissions/pollutants; pollutant emissions from natural (biogenic) sources; source-receptor relationships (understanding where pollutants come from); ultraviolet radiation |

# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Health and Environmental Effects Research Laboratory
### Areas of Expertise

| | Telephone | Areas of Expertise |
|---|---|---|
| *Office of the Director* | | |
| Lawrence W. Reiter, Director | 919-541-2281 | Environmental health and neurotoxicology |
| *Research Planning and Coordination Team* | | |
| Robert S. Dyer | 919-541-2760 | Strategic planning |
| John J. Vandenberg | 919-541-4527 | Health and ecological effects of air pollutants |
| Fred S. Hauch | 919-541-3893 | Health and ecological effects of water pollutants |
| Sue R. McMaster | 919-541-3844 | Health and ecological effects of pesticides and toxics |
| John J. Vandenberg | 919-541-4527 | Health and ecological effects of air pollutants |
| Michael D. Waters | 919-541-2537 | International research activities |
| *Office of the Associate Director for Health* | | |
| Harold Zenick, Associate Director | 919-541-2283 | Noncancer risk assessment with special emphasis on reproductive risk assessment; environmental justice; U.S.-Mexico border environmental health |
| Joe A. Elder | 919-541-2542 | Radio frequency (RF) radiation; electric and magnetic fields |
| **Environmental Carcinogenesis Division** | | |
| Larry D. Claxton, Director | 919-541-2329 | Cancer research; complex mixtures |
| *Biochemistry and Pathology Branch* | | |
| Stephen Nesnow, Chief | 919-541-3847 | Carcinogenicity mechanisms |
| Carl Blackman | 919-541-2543 | Cellular communication |
| Anthony DeAngelo | 919-541-2568 | Whole animal carcinogenicity |
| Leon King | 919-541-0720 | DNA adducts; nitroarene metabolism |
| Kirk Kitchin | 919-541-7502 | Biochemistry |
| Marc Mass | 919-541-3514 | Oncogenes |
| James Rabinowitz | 919-541-5714 | Computational chemistry |
| Ann Richard | 919-541-3934 | Structure activity relationships (SAR) and carcinogenicity |
| Jeffery Ross | 919-541-2974 | DNA adducts and polycyclic aromatic hydrocarbons (PAHs) |
| *Genetic and Cellular Toxicology Branch* | | |
| Martha Moore, Chief | 919-541-3933 | Genotoxicity |
| James Allen | 919-541-4778 | Cytogenetics |
| Karen Brock | 919-541-3080 | Genotoxicity |
| David DeMarini | 919-541-1510 | Mutation spectra |
| James Fuscoe | 919-541-3918 | Molecular mechanism of carcinogenicity |

(continued)

# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Health and Environmental Effects Research Laboratory
### Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| Susan George | 919-541-5036 | Gastrointestinal and pulmonary tract toxicology |
| Andrew Kligerman | 919-541-4254 | Cytogenetics |
| **Experimental Toxicology Division** | | |
| Linda S. Birnbaum, Director | 919-541-2655 | Pharmacokinetics; toxicology |
| *Pulmonary Toxicology Branch* | | |
| Daniel L. Costa, Chief | 919-541-2655 | Pulmonary toxicology; physiology |
| Kevin L. Dreher | 919-541-3691 | Molecular and cellular biologist; pulmonary inflammation and fibrosis; pulmonary adaptation to environmental injury; transgenic animal models |
| Jan A. Dye | 919-541-0678 | Pulmonary cell biology; pulmonary medicine and infectious disease syndromes; pulmonary function testing |
| Stephen H. Gavett | 919-541-2555 | Assessment of airway reactivity; cytokine regulation of inflammatory responses and influence on physiology; allergy and asthma; inhalation exposure systems and technology |
| Gary E. Hatch | 919-541-2658 | Age/diet susceptibility; oxidative injury; antioxidant defenses; biochemical toxicology |
| Urmila P. Kodavanti | 919-541-4963 | Nucleic acid isolation; polymerase chain reaction (PCR); western blotting; immunohistochemistry |
| Ted B. Martonen | 919-541-7875 | Aerosol science |
| John H. Overton | 919-541-5715 | Mathematical/computer modeling |
| William P. Watkinson | 919-541-4018 | Rodent electrocardiography; mammalian thermoregulation; radiotelemetry methodology |
| Jean M. Wiester | 919-541-7738 | Pulmonary toxicology; physiology |
| *Pharmacokinetics Branch* | | |
| James D. McKinney, Chief | 919-541-1498 | Molecular toxicology |
| John W. Allis | 919-541-2632 | Chemistry; biochemical toxicology |
| Mike J. DeVito | 919-541-0061 | Toxicology; pharmacokinetics; halogenated aromatic hydrocarbons |
| Janet J. Diliberto | 919-541-7921 | Toxicology; pharmacokinetics; dioxins and related compounds |
| Marina V. Evans | 919-541-0838 | Pharmacokinetics modeling; sensitivity analysis |
| Larry L. Hall | 919-541-2774 | Pharmacology; toxicology |

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## National Health and Environmental Effects Research Laboratory
## Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| Mike F. Hughes | 919-541-2160 | Toxicology; drug metabolism |
| Elaina M. Kenyon | 919-541-0043 | Toxicology and benzene |
| Rex A. Pegram | 919-541-0410 | Toxicology; pharmacokinetics; drinking water; disinfection byproducts |
| Jane Ellen Simmons | 919-541-7829 | Mixtures/interaction toxicology |
| David J. Thomas | 919-541-4974 | Metals/biochemical toxicology |
| *Immunotoxicology Branch* MaryJane Selgrade, Chief | 919-541-2657 | Immunotoxicology |
| Robert W. Luebke | 919-541-3672 | Immunotoxicology; parasitology |
| Lisa K. Ryan | 919-541-2592 | Endotoxin; air pollutant particulates; UV light; cytokines; macrophage; biology; influenza; host defense; mechanisms; pulmonary immunology |
| Denise M. Sailstad | 919-541-2545 | Contact hypersensitivity; ultraviolet radiation effects; enzyme-linked immunosorbent assay (ELISA) development; immunotoxicology |
| Ralph J. Smialowicz | 919-541-5776 | Immunotoxicology; immunology; bacteriology |
| **Human Studies Division** | | |
| *Office of the Director* Hillel Koren, Director | 919-966-6200 | Respiratory human disease; controlled exposure studies |
| George Goldstein | 919-966-6204 | Measurement of eye irritation |
| John Kinsey | 919-966-6209 | Engineering related to environmental pollutant exposure in both ambient and clinical settings; particle physics, deposition and concentration |
| Elston Seal | 919-966-6217 | Environmental medicine; response of sensitive populations to air pollutants; human research subject; human right ethics |
| *Clinical Research Branch* Robert Devlin, Acting Chief | 919-966-6255 | Molecular biology; pulmonary injury |
| Vernon Benignus | 919-966-6242 | Neurotoxicology; human exposures; modeling |
| Howard Kehrl | 919-966-6208 | Pulmonary medicine; asthma; airway reactivity; sensitive subpopulations; inhalation toxicology; multiple chemical sensitivity |
| Chong Kim | 919-966-5049 | Deposition of gases and particles in human lung; clearance of gases and particles from human lung |

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# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Health and Environmental Effects Research Laboratory
### Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| Bill McDonnell | 919-966-6220 | Health effects of ozone exposure |
| Mike Madden | 919-966-6257 | Cell biology related to reactive oxygen species generation; lipid metabolism; DNA damage; human physiology related to air pollution toxicology, primarily ozone, air toxics, and $PM_{10}$ |
| David Otto | 919-966-6226 | Neurotoxicity testing of children and adults; health assessment of lead exposure; health assessment of indoor air pollutants |
| *Epidemiology and Biomarkers Branch* | | |
| Rebecca Calderon, Acting Chief | 919-966-0617 | Epidemiology; drinking water; infectious disease |
| Dina Schreinemachers | 919-966-5875 | Statistical analysis of data from epidemiological studies |
| **Neurotoxicology Division** | | |
| Hugh A. Tilson, Director | 919-541-2671 | Developmental neurotoxicology; polychlorinated biphenyls (PCBs) |
| *Neurobehavioral Toxicology Branch* | | |
| Robert MacPhail, Chief | 919-541-7833 | Behavioral toxicology; pesticides |
| Philip Bushnell | 919-541-7747 | Attention; cognition; solvents |
| Kevin Crofton | 919-541-2672 | Auditory function; solvents; PCBs |
| Chris Gordon | 919-541 1509 | Thermoregulation; pesticides |
| Virginia Moser | 919-541-5075 | Behavioral screening; pesticides |
| Mark Stanton | 919-541-7783 | Developmental neurotoxicology |
| *Neurophysiological Toxicology Branch* | | |
| William Boyes, Chief | 919-541-7538 | Sensory function; solvents, pesticides |
| David Herr | 919-541-0380 | Vision; solvents |
| Kenneth Hudnell | 919-541-7866 | Sensory function, humans |
| Diane Miller | 919-541-4186 | Stress; endocrine dysfunction |
| Tim Shafer | 919-541-0647 | Channel function; metals |
| *Cellular and Molecular Toxicology Branch* | | |
| Hugh Tilson, Acting Chief | 919-541-2671 | Developmental neurotoxicology; PCBs |
| Stanley Barone | 919-541-3916 | Developmental neuroanatomy; $CH_3Hg$ |
| Karl Jensen | 919-541-1560 | Neuroanatomy; pesticides |
| William Mundy | 919-541-7725 | Neurochemistry; metals |
| James O'Callaghan | 919-541-7779 | Neurochemistry; neural markers |
| Stephanie Padilla | 919-541-3956 | Neurochemistry; cholinesterase inhibitors |
| Prasada Kodavanti | 919-541-7584 | Neurochemistry; calcium; PCBs |
| Bellina Veronesi | 919-541-5780 | Neuroimmunology |

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## National Health and Environmental Effects Research Laboratory
## Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| **Research and Administrative Support Division** | | |
| Barry Howard, Acting Director | 919-541-2729 | |
| *Biometry Branch* | | |
| John Creason | 919-541-2598 | Biostatistics |
| Don Doerfler | 919-541-7741 | Biostatistics |
| Dennis House | 919-541-2389 | Biostatistics |
| Jerry Highfill | 919-541-4068 | Biostatistics |
| Judith Schmid | 919-541-0486 | Biostatistics |
| Woodrow Setzer | 919-541-0128 | Biostatistics |
| *Special Studies and Technical Support Branch* | | |
| Richard Linko | 919-541-4279 | Laboratory animal resources |
| Michael Ray | 919-966-0625 | Quality assurance (QA) for clinical research; environmental pollutant exposures |
| *Federal Technology Transfer Act / National Technology Transfer and Advancement Act Agreements* | | |
| Ron Rogers | 919-541-2370 | Technology transfer coordinator |
| Virginia Houk | 919-541-2815 | Automation of mutagenicity assays |
| MaryJane Selgrade | 919-541-2657 | Development of polyclonal antibodies to rat cytokines |
| Ralph Cooper | 919-541-4084 | Development of methods to evaluate effect of herbicides on endocrine and reproductive function |
| **Reproductive Toxicology Division** | | |
| *Office of the Director* | | |
| Robert J. Kovlock, Director | 919-541-2326 | Developmental biology; endocrine disruptors |
| *Development Biology Branch* | | |
| John M. Rogers, Chief | 919-541-5177 | Developmental toxicity |
| Barbara Abbott | 919-541-2753 | Developmental toxicity of dioxin |
| James Andrews | 919-541-2487 | *In vitro* teratology |
| Neil Chernoff | 919-541-2651 | Teratology |
| Frank Copeland | 919-541-2678 | Metabolism |
| Phil Hartig | 919-541-0492 | Molecular biology |
| Sid Hunter | 919-541-3490 | Mechanism of teratogenicity |
| Clint Kawanishi | 919-541-7965 | Molecular biology |
| Ed Massaro | 919-541-3177 | Mechanisms of cytotoxicity |
| Leonard Mole | 919-541-2680 | Analytical chemistry |

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# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Health and Environmental Effects Research Laboratory

### Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| *Endocrinology Branch* | | |
| Ralph Cooper, Chief | 919-541-4084 | Neuroendocrinology |
| Audrey Cummings | 919-541-5194 | Pregnancy and uterine function |
| Jerome Goldman | 919-541-2320 | Hormonal control of ovulation |
| Earl L. Gray | 919-541-7750 | Developmental reproductive toxicity |
| William Kelce | 919-541-1580 | Steroid receptor biochemistry |
| Chris Lau | 919-541-5097 | Mechanisms of developmental toxicity |
| Susan Laws | 919-541-0173 | Receptor biochemistry |
| Mike Narotsky | 919-541-0591 | Developmental toxicology; hormonal control of pregnancy |
| *Gamete and Early Embryo Biology Branch* | | |
| Sally Darney, Chief | 919-541-3826 | Gamete biology |
| David Dix | 919-541-2701 | Stress proteins |
| Ken Elstein | 919-541-3581 | Flow cytometry |
| Gary Held | 919-541-0286 | Molecular biology |
| Gary Klinefelter | 919-541-5779 | Reproductive biology/toxicology |
| Jeff Welch | 919-541-0513 | Reproductive biology; spermatogenesis |
| Robert Zucker | 919-541-1585 | Flow cytometry |
| *Office of the Associate Director for Ecology* | | |
| Gilman Veith, Associate Director | 919-541-4130 | Structure-activity relationships |
| Shabeg Sandhu | 919-541-3850 | Ecogenetic toxicology; population genetics |
| Laura Jackson | 919-541-3088 | Ecological indicators; landscape analyses; EMAP |
| Jennifer Orme Zavaleta | 919-541-3558 | Risk assessments/risk characterization |

# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Risk Management Research Laboratory

### Areas of Expertise

| | Telephone | Areas of Expertise |
|---|---|---|
| **Air Pollution Prevention and Control Division** | | |
| *Office of the Director* | | |
| Frank T. Princiotta, Director | 919-541-2821 | Air and energy environmental assessment and control technology |
| Doug McKinney | 919-541-3006 | Air research planning |
| G. Blair Martin | 919-541-7504 | Combustion, incineration; furnace injection for $SO_x$ control |
| Michael Maxwell | 919-541-3091 | International control technology |
| *Technical Services Branch* | | |
| Wade Ponder, Chief | 919-541-2818 | Flue gas desulfurization; control technology; pollution prevention; conventional combustion environmental assessment |
| Nancy Adams | 919-541-5510 | Quality assurance/quality control audits; environmental toxicology; pesticide effects |
| Jeff Ryan | 919-541-1437 | Dioxin/organics measurement; source/stack sampling methodology |
| Richard Shores | 919-541-4983 | Environmental engineering; instrumentation for ambient air monitoring; QA/QC field audit programs |
| Shirley Wasson | 919-541-1439 | X-ray fluorescence; x-ray diffraction; scanning electron microscopy; QA/QC auditor; metals analysis |
| *Air Pollution Technology Branch* | | |
| Robert E. Hall, Chief | 919-541-2477 | Combustion modification control technology; fundamental hazardous waste incineration research |
| Theodore Brna | 919-541-2684 | Flue gas cleaning; chlorofluorocarbons alternatives; power plant cooling systems; property evaluation of refrigerants and lubricants |
| Brian Gullett | 919-541-1534 | Formation and prevention of chlorinated organics from incineration processes; sorption of mercury from industrial processes |
| Norm Kaplan | 919-541-2556 | Integrated air pollution control system cost model; economic evaluations of $SO_2$, $NO_x$, particulate matter control |
| Jim Kilgroe | 919-541-2854 | Municipal solid waste combustion; hazardous waste combustion; formation and destruction of polychlorinated dibenzo-dioxin/polychlorinated dibenzo-furan |
| David Lachapelle | 919-541-3444 | Combustion modification control technology; $NO_x/SO_x$ control |

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# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Risk Management Research Laboratory

### Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| C. W. Lee | 919-541-7663 | Chlorofluorocarbons and electrical industrial waste incineration; biomass combustion |
| Paul Lemieux | 919-541-0962 | Products of incomplete combustion from incineration; artificial intelligence for combustion control; tire burning; emergency safety vents |
| Bill Linak | 919-541-5792 | Toxic metal transformation/aerosol formation during hazardous and municipal waste incineration |
| Andy Miller | 919-541-2920 | $NO_x$, air toxics, use of artificial intelligence for combustion applications |
| Sam Rakes | 919-541-2828 | $SO_x$ control technology |
| Charles Sedman | 919-541-7700 | Flue gas cleaning technology |
| Jack Wasser | 919-541-2476 | $NO_x$ and particulates from stationary diesel engines and gas turbines, industrial boilers, woodstoves and industrial furnaces and processes; hazardous waste incineration in fluidized bed combustors |
| *Atmospheric Protection Branch* | | |
| William J. Rhodes, Chief | 919 541-2853 | Emissions and mitigation for global climate change, e.g., biomass, greenhouse gases, ozone depleting substances |
| Evelyn Baskin | 919-541-2429 | Refrigeration/heat transfer/thermosciences (ozone depleting substances and biomass research) |
| Lee L. Beck | 919-541-0617 | Emissions and mitigation software related to global climate change |
| Robert H Borgwardt | 919-541-2336 | Mitigation technology for greenhouse gas emissions |
| Cynthia L. Gage | 919-541-0590 | Emissions and mitigation for global climate change (particularly ozone depleting substances) |
| Robert V. Hendriks | 919-541-3928 | Refrigeration technologies and biomass utilization |
| James Jetter | 919-541-4830 | Emissions and mitigation for global climate change; refrigeration systems; automotive air conditioning |
| David A. Kirchgessner | 919 541-4021 | Methane emissions, especially coal mines; natural gas processing; petroleum |
| Carol Purvis | 919-541-7519 | Small biomass-to-electricity technologies |
| N. Dean Smith | 919-541-2708 | Alternative chemicals for pollution prevention, alternatives for greenhouse gases and ozone depleting substances |

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## National Risk Management Research Laboratory
## Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| Ronald J. Spiegel | 919-541-7542 | Mitigation for global climate change, e.g., fuel cells; advanced control systems; photovoltaic systems |
| Susan Thorneloe | 919-541-2709 | Emissions and mitigation for waste management; small-scale combustion devices; large area sources; evaluation of integrated waste management strategies using life-cycle assessment principles |
| *Emissions Characterization and Prevention Branch* | | |
| Larry Jones, Chief | 919-541-7716 | Emission characterization methodologies; projection models; field validation of improved methods |
| Chuck Darvin | 919-541-7633 | Pollution prevention methodologies (spraybooths, cleaning) |
| Chris Geron | 919-541-4639 | Biogenic emissions characterization |
| Bruce Harris | 919-541-7907 | Particulate heavy duty mobile emissions characterization |
| Julian Jones | 919-541-2489 | Toxic air emissions characterization |
| Sue Kimbrough | 919-541-2612 | Emissions modeling |
| Mike Kosusko | 919-541-2734 | Pollution prevention methodologies (general) |
| Robert McCrillis | 919-541-2733 | Particulate and pollution prevention methodologies (coatings) |
| Chuck Mann | 919-541-4593 | Stationary area source emissions characterization |
| Chuck Masser | 919-541-7586 | Particulate and volatile organic carbon emissions characterization |
| Carlos Nuñez | 919-541-1156 | Pollution prevention methodologies (general) |
| Geddes Ramsey | 919-541-7963 | Particulate and pollution prevention (coatings) |
| Ted Ripberger | 919-541-2924 | Light duty mobile emissions characterization |
| Niranjan Vescio | 919-541-0487 | Remote sensing of mobile source emissions |
| Chester Vogel | 919-541-2827 | Pollution prevention methodologies (adhesives) |
| Kaye Whitfield | 919-541-2509 | Pollution prevention methodologies (paint stripping) |
| *Indoor Environment Management Branch* | | |
| Michael Osborne, Chief | 919-541-4113 | Indoor air pollutant source/emissions characterization; indoor air quality mitigation; radon mitigation |
| John C. S. Chang | 919-541-3747 | Biocontaminants; volatile organic carbon source/sink characterization; volatile organic carbon emissions modeling |

(continued)

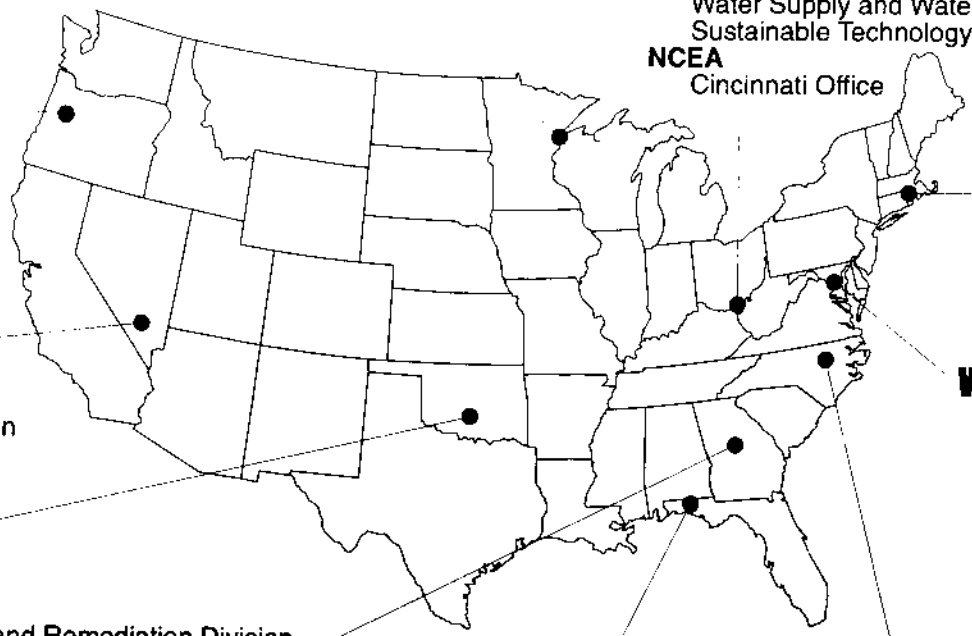
# Office of Research and Development Technical Contacts

## *Research Triangle Park, North Carolina*

### National Risk Management Research Laboratory
### Areas of Expertise (continued)

| | Telephone | Areas of Expertise |
|---|---|---|
| D. Bruce Henschel | 919-541-4112 | Cost analysis of indoor air quality control techniques; building energy modeling; radon reduction in existing houses |
| Betsy M. Howard | 919-541-7915 | Pollution prevention; particle board; large chamber testing; conversion varnishes |
| Russell N. Kulp | 919-541-7980 | Ventilation systems (large building studies; air duct cleaning; heating; ventilation and air conditioning pollution sources; gas indoor air phase filtration; energy and indoor air quality studies) |
| Kelly W. Leovic | 919-541-7717 | Pollution prevention (office equipment, aerosol consumer products, engineered wood products); technology transfer |
| Mark A. Mason | 919-541-4835 | Bioresponse methods development; chemical source characterization; large chamber testing |
| Marc Y. Menetrez | 919-541-7981 | Large building measurements (indoor air quality), ventilation, building dynamics; heating, ventilation and air conditioning, diagnostic strategy |
| Ronald B. Mosley | 919-541-7865 | Indoor air pollutants originating in soil; mathematical modeling, indoor particles; soil contaminants |
| Richard B. Perry | 919-541-2721 | Radon diffusion measurement; test method development; ventilation systems research |
| David C. Sanchez | 919-541-2979 | Radon research (measurement, transport modeling, building dynamics, new construction standards); indoor air quality; diffusion barrier testing; radon-free schools |
| Leslie E. Sparks | 919-541-245 | Indoor air quality and exposure modeling; air cleaners; indoor particles |
| Raymond S. Steiber | 919-541-2288 | Indoor air quality particulate and chemistry studies (indoor ozone, biological volatile organic carbons, indoor particulate, remedial device testing, marker compounds) |
| W. Gene Tucker | 919-541-2746 | Control of indoor air quality; ASHRAE Standard 62; bioresponse methods; source emissions; indoor/outdoor particles |
| James B. White | 919-541-1189 | Low-emitting/low-impact sources; indoor air quality emission source database; indoor air quality and life cycle assessment; environmental resources guide; facilities design and operation; CADD-based life cycle analysis for indoor air quality; textiles |

# Locations of ORD Laboratories and Centers



**Duluth, MN**
**NHEERL**
Mid-Continent Ecology Division

**Cincinnati, OH**
**NERL**
Ecological Exposure Research Division
Human Exposure Research Division
**NRMRL**
Land Remediation and Pollution Control Division
Technology Transfer and Support Division
Water Supply and Water Resources Division
Sustainable Technology Division
**NCEA**
Cincinnati Office

**Corvallis, OR**
**NHEERL**
Western Ecology Division

**Narragansett, RI**
**NHEERL**
Atlantic Ecology Division

**Las Vegas, NV**
**NERL**
Characterization Research Division

**Washington, DC**
**NCEA**
**NCERQA**
**NRMRL**
**Assistant Administrator**
**Office of Resources Management and Administration**
**Office of Science Policy**

**Ada, OK**
**NRMRL**
Subsurface Protection and Remediation Division

**Athens, GA**
**NERL**
Ecosystems Research Division

**Gulf Breeze, FL**
**NHEERL**
Gulf Ecology Division

**Research Triangle Park, NC**
**NERL**
Air Exposure Research Division
Air Measurements Research Division
Atmospheric Modeling Division
Atmospheric Processes Research Division
**NHEERL**
Environmental Carcinogenesis Division
Human Studies Division
Neurotoxicology Division
Reproductive Toxicology Division
Experimental Toxicology Division
**NRMRL**
Air Pollution Prevention and Control Division
**NCEA**
Research Triangle Park Office

| | |
|---|---|
| **NCEA** | National Center for Environmental Assessment |
| **NCERQA** | National Center for Environmental Research and Quality Assurance |
| **NERL** | National Exposure Research Laboratory |
| **NHEERL** | National Health and Environmental Effects Research Laboratory |
| **NRMRL** | National Risk Management Research Laboratory |

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