National Institute of Environmental Health Sciences | **Superfund Basic Research Program**

A LEGACY IN MULTIDISCIPLINARY RESEARCH

Charting a Course for Advancing Basic Research to Practice

VOLUME 3

SBRP Contributions

U.S. Department of Health and Human Services | National Institutes of Health | National Institute of Environmental Health Sciences

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SBRP Contributions

2003 SBRP External Advisory Group

A Working Group of the National Environmental Health Sciences Council

Research Triangle Park, N.C.

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A LEGACY IN MULTIDISCIPLINARY RESEARCH

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4a. Overview of SBRP Contributions

This section is intended to demonstrate the contributions of the SBRP. Whereas Section 3 is primarily a listing of SBRP projects by topic, Section 4 highlights how a multidisciplinary approach to science, as exemplified by the SBRP, can contribute to improved public health. Such contributions are accomplished by providing sound science to those making public policy, regulatory, remediation and risk reduction decisions.

While the entirety of the SBRP's research is conducted under the rubric of improving public health, we wanted to frame the SBRP research in a manner that allows you to view the SBRP science in context of its potential application in the decision-making processes. With this in mind, the materials in this section have been grouped into the following categories:

- Contributions to the Regulatory Decision-Making Process
- Contributions to Public Health
- Contributions to Understanding Mechanisms of Toxicity of Superfund Chemicals
- Contributions to the Development and Advancement of Remediation Processes
- Contributions to Human and Ecological Risk Assessments Processes
- Innovative Spinoffs
- Contributions of Outreach Programs
- Success Stories: SBRP-Funded Graduate Students and Post-Doctoral Fellows
- Contributions of Multidisciplinary Research

In an attempt to present what the SBRP Program Directors consider to be the most significant contributions from their program, we requested that they submit a summary of their program's most "Notable Advances" for the entire period they have received SBRP funding. Their submissions constitute the majority of Section 4. We did exercise some discretion in selecting those accomplishments that we judged to be representative of the diversity of scientific creativity and public health advancement. You will find that while the material presented in this section is extensive, it is important to realize that it does not represent all of the important research findings that have emanated from the Program.

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4b. Potential Discussion Questions

Again, as in Section 3, below are the (same) questions that you may wish to consider as you assess the contributions the SBRP has made to advance science to society's benefit and improve public health.

1. Has the SBRP made a contribution to the advancement of science in these topic areas? (Or is the information provided inadequate to assess the contributions? If so, can we provide you more specific information to help you?)
2. Are there critical research areas that have been overlooked, or that need priority attention?
3. Within a topic area, are there specific aspects that should receive less emphasis? Why? (Given that we have limited resources, it would be beneficial to know if there are lower priority areas to offset the other higher priority areas.)
4. Based on the state of the science in the topic areas, are there major areas where concentrated effort is more likely to produce "breakthroughs" that would more dramatically advance our understanding of the field; limit exposures at hazardous waste sites; or improve public health?
5. Can we do a better job at communicating the results of the science and its potential implications to the general public and other involved stakeholders? If so, are there specific examples or approaches that we could use as models for communicating research results?
6. In particular, how can we communicate our research findings to the broader community of state site managers and professionals who are making decisions at hundreds of small or large hazardous waste sites around the country?
7. Are there other partnerships or venues that should receive priority attention by the SBRP in communicating its research results?
8. Are there additional outreach opportunities that we should explore for involving local communities or tribes in the understanding and application of our research results?
9. Are our efforts in multidisciplinary training effective? Are they filling a societal need? If so, is there anything else that we should be doing to advocate for multidisciplinary training?

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4c. Contributions to Decision-Making Processes

Although the SBRP has a mandate to conduct fundamental research, we recognize that to fully achieve our goals of supporting the national Superfund program, use-oriented research must play a primary role in our research strategy. The Program's research must be timely and applicable to very real environmental problems. The summaries in this section demonstrate that our investments are responsive to this need and contribute to the decision-making process.

For example, over the last couple of years two of the major environmental issues have been the lowering of the arsenic drinking water standard and the decision to dredge the Hudson River for PCBs. It is not surprising that work conducted by SBRP-supported investigators was included in these decision-making processes. Not only was their data taken into consideration, but their expertise was called on to contribute to the dialogue.

These are not the only cases where Program investigators have served as advisors. Most recently, three investigators were invited to serve on the USEPA Contaminated Sediments Science Panel. This panel is charged with developing a research and management agenda for reducing the risk of contaminated soils to humans and the ecosystem. Involvement of qualified, dedicated professionals on advisory panels such as these ensures that decisions are based on the best available science.

The following four summaries illustrate how the SBRP has contributed to important national environmental issues. Clearly, other research efforts fall into this category, but as we note in the Overview, we have been selective in the materials we include in each section. Furthermore, we only have included a research summary in one topic area, even though it may apply to other topics (e.g. Wildlife Applications to Remediation Decision Making is included in "Contributions to Human and Ecological Risk Assessment Processes"; however, it also fits in this section.)

4c.i Understanding the Mechanisms of Arsenic-Induced Cancer and Vascular Disease

The USEPA's longstanding drinking water standard for arsenic (50 ppb) was lowered to 10 ppb in 2001 following several years of intense scientific and public policy debate. The early results of the Dartmouth epidemiology study – which is the only United States population-based case-control study linking individual arsenic exposures (measured in toenail samples) with cancer risk – was one of the key studies that factored into that debate. The 1999 and 2000 National Research Council (NRC) reports on arsenic that the USEPA used as a scientific basis for lowering the drinking water standard also cited the results of two Dartmouth SBRP molecular toxicology groups. One group, led by Aaron Barchowsky, demonstrated that very low levels of arsenic (in the range equivalent to elevated United States drinking water values) altered blood vessel growth as a result of altering

critical cell signaling events of both the epithelial and endothelial cells that form the blood vessels. This has implications both for vascular disease and for metastatic cancer. The other group, led by Joshua Hamilton, made the unexpected discovery that arsenic at very low levels is a potent endocrine disruptor, effectively blocking the ability of steroid hormones such as estrogen, testosterone and corticosteroids to regulate the behavior of cells through their receptors. Such endocrine disruption could explain, at least in part, how arsenic is able to increase risk of various types of cancer as well as vascular disease, cardiovascular disease, diabetes, and reproductive and developmental effects. *This latter study was cited in Congressional testimony by Christine Todd Whitman, head of the USEPA, as one of the key new studies that supported lowering the arsenic drinking water standard to protect the health of the U.S. population.*

The Dartmouth SBRP program continues to play a prominent role in this public health debate. For example, Joshua Hamilton served as an external reviewer to the NRC's 2000 Arsenic Report that was used to set the new drinking water standard, and Margaret Karagas served on several review panels on arsenic and health effects, most recently on the World Health Organization's International Agency for Research on Cancer (IARC) update of their monograph on arsenic and cancer.

[**Hamilton/Barchowsky (Dartmouth)**]

4cii Arsenic Biomarker Epidemiology

The revelation that arsenic is a major environmental carcinogen is largely due to the work of epidemiologist Allan Smith's group at the University of California-Berkeley (UC-B) SBRP program. This research provided definitive evidence that arsenic in drinking water causes bladder, lung, and other internal cancers. Their arsenic research activities began more than 10 years ago with a risk assessment focusing mainly on cancer. This work, co-authored by the UC-B SBRP Program Director Martyn Smith, led to the conclusion that the cancer risks from inorganic arsenic in drinking water were potentially very high. The Arsenic Health Effects Research Program was then started with funding from the SBRP to study the health risks from exposure to inorganic arsenic. Currently it involves international research projects including studies and investigations in Argentina, Chile, India, Bangladesh as well as projects in California and Nevada in the United States.

Employing novel biomarkers developed in Martyn Smith's laboratory, the research team showed that effects on bladder epithelium occur at or around the U.S. drinking water standard of 50 ppb. The SBRP-sponsored epidemiological studies in Nevada, Argentina, and Chile, made clear the need for better understanding of arsenic-related cancers, and led to additional funding to investigate the dose-response relationship in greater detail.

Allan Smith and John Wiencke both serve on the National Academy of Sciences Subcommittee on Arsenic in Drinking Water set up by the Board on Environmental Studies and Toxicology, National Research Council. Dr. Smith has also served as a Consultant to the World Health Organization on the arsenic problems in Bangladesh including five visits to Bangladesh and preparation of three reports.

This research has reduced the uncertainty in the risk assessment for arsenic and impacted the regulation of this important environmental contaminant. To summarize, SBRP-funded accomplishments:

- Provided definitive evidence (from studies conducted in Argentina and Chile) that arsenic is a potent cause of human bladder cancer.
- Provided definitive evidence (from studies conducted in Argentina and Chile) that arsenic in drinking water is a potent cause of human lung cancer.
- Demonstrated that epidemiological and experimental human data do not support that arsenic methylation protects from low dose effects.
- Showed that with exposure to water containing around 600 ug/L, 1 in 10 adult deaths may be due to arsenic-caused cancers, the highest environmental cancer risk ever reported.
- Identified a dose-response relationship between arsenic exposure and bladder cell micronuclei, a genotoxic biomarker of early effect.
- Identified a preliminary dose-response relationship between arsenic concentration in well water in India and the occurrence of keratoses and hyperpigmentation.
- Studies currently under way in India, Chile and the United States will allow projection of cancer risks with individual exposure data.
- Studies are currently under way to identify nutritional and genetic susceptibility to arsenic effects.

[**Smith (University of California – Berkeley)**]

4c.iii Persistent Organochlorines in the Hudson River Watershed

A major accomplishment of the Mount Sinai SBRP has been development of the most highly detailed analysis to date of chemical pollutants in the Hudson River and its watershed. Time trends in pollutant levels, rates of pollutant degradation *in situ*, geographical patterns of pollutant distribution and identification of sources are all included within this comprehensive state-of-the-art database. Dated sediment cores have been the principal source of information in this project, and the Mount Sinai SBRP program has been the major proponent of the use of dated sediment samples to study the sources, transport and fate of persistent contaminants.

The defining characteristic of the Mount Sinai SBRP approach to the study of persistent contaminants in the Hudson River watershed has been the use of a multi-year, multi-contaminant perspective. Specific results of this research include the following:

- Samples collected to define the temporal and geographic distribution of PCBs in the Upper Hudson revealed major trace metal contamination from a pigment plant, and significant dioxin contamination from poorly characterized sources.
- Samples collected to trace the dioxin contamination from the 80 Lister Avenue Superfund site revealed significant sources of chlordane in the Passaic and Hackensack basins, and major point sources of mercury in the western NY/NJ harbor.
- Sediment cores from Central Park Lake that defined the history of atmospheric deposition of lead and other trace metals to the NY Metropolitan area are now being studied to determine fluxes of dioxins, PCBs, and PAHs.
- Archived samples, collected as far back as the 1970s are being analyzed to define the basin-wide temporal and geographic distribution of alkylphenol ethoxylates – a class of endocrine-disrupting compounds found in detergents. The use of these compounds has been restricted in much of Europe, but continues to increase in the United States. This series of analyses will permit the Mount Sinai researcher to examine time trends and geographic distribution.

The World Trade Center disaster has been an especially acute focus of the Mount Sinai research since September 11, 2001. A research team led by Richard Bopp has established several harbor sediment sampling sites within a few miles of Ground Zero as an extension of the ongoing assessment of the chronology of sediment contamination. Dr. Bopp's team is now in the process of comparing the results of analyses of samples collected at these sites in October 2001 with data from pre-9/11 archived samples. This comparison will provide a direct measure of the effect of the disaster. The "contaminants of interest" in the samples near Ground Zero will be expanded beyond PCBs and chlorinated dioxins and furans to include PBDEs (common fire retardant chemicals), brominated dioxins and furans (high temperature transformation products of PBDEs) and PAHs (jet fuel indicators).

Federal, state and local officials have all come to rely on the geochemical data from the Mount Sinai SBRP as an honest, unbiased and scientifically accurate source of critical information for major regulatory decisions involving the Hudson River Superfund site. *These data helped to reduce uncertainty and guide good judgment in the USEPA decision to require dredging of contaminated sediments from certain areas of the upper Hudson. Indeed, the data from Mt. Sinai's SBRP program helped to define the dredging "hot spots" in the River.* Similarly in the next several years, the Mount Sinai researchers anticipate that these geochemical findings coupled with data from their epidemiologic studies will help guide upcoming decisions on whether to reopen the centuries' old Hudson River commercial striped bass fishery, which has been closed since 1976 because of PCB contamination. These data and interpretations have also played a major role in determining the extent and severity of dioxin contamination from the 80 Lister Avenue Superfund Site on the Lower Passaic River. Furthermore, the recent partnership of Mount Sinai SBRP-supported researchers with the NYS Department of Environmental Conservation, the USEPA, and the National Oceanic

and Atmospheric Administration is exploring the possibility of Natural Resource Damage claims involving Hudson River Superfund sites.

[**Bopp (Mount Sinai School of Medicine)**]

4c.iv Developmental Neurotoxicity of Chlorpyrifos: Mechanism and Consequences

Duke University SBRP researchers led by Ted Slotkin are studying the most widely used organophosphate insecticide, chlorpyrifos, evaluating both the mechanisms underlying its developmental neurotoxicity and the critical periods delineating developmental vulnerability. The widespread use of chlorpyrifos has raised concern about the potential consequences of fetal and childhood exposure. Slotkin's research team has completed studies on the mechanisms by which chlorpyrifos disrupts brain development, focusing on events at the level of the basic processes of cell division and differentiation. In addition, they are exploring non-mammalian test systems that might permit rapid detection of neurotoxicity. The research spans all levels of organization, from the cellular and molecular events underlying the toxic mechanisms, through the morphological assembly of the nervous system and the eventual behavioral outcomes.

Results of the studies at Duke point to a number of important features of this toxicity:

- The vulnerable period extends into stages of brain development corresponding to early childhood, a period in which high exposures have been reported as a result of home or garden application.
- The mainstay of toxicity testing, cholinesterase inhibition, may be totally inadequate to predict the developmental neurotoxicity of chlorpyrifos.
- The research team has identified a number of candidate processes that may serve as surrogate markers of developmental toxicity, in lieu of cholinesterase, and is in the process of devising cell culture and invertebrate models for the rapid screening of these markers.
- The Duke scientists have begun to draw the causal connections between events at the molecular and biochemical level, and the resultant behavioral anomalies attributable to chlorpyrifos.
- The introduction of non-mammalian and cell culture models will enable the development of high-throughput, rapid screening techniques to determine whether other candidate compounds are likely to be developmental neurotoxins. Slotkin has already validated this approach with studies of other organophosphates, organochlorines and carbamates. These screens should be able to incorporate toxicogenomics, proteomics, and other novel screening techniques, much more readily than cumbersome, costly testing with animal models.

This work has had significant impact on public policy and on the scientific community. *The findings of this project were cited in the USEPA's decision to restrict domestic use of chlorpyrifos, and subsequently to withdraw registration of this pesticide.* Subsequent USEPA documents on their website

(for example, the 2002 reassessment of developmental neurotoxicity of organophosphates) cite this SBRP work heavily.

Finally, it is critical to note that nerve gases are organophosphates, so that these findings are applicable to exposures from bioterrorism.

[**Slotkin (Duke University)**]

4d. Contributions to Public Health

As stated in the Overview, we believe that the overarching goal of SBRP-funded research is to improve public health. While each of the areas presented in Section 4 support this goal, the examples provided on the following pages demonstrate the potential direct application of the research to the public health arena. Here we highlight some of our research efforts that can be used by the professionals directly responsible for promoting public health: the ATSDR, USEPA, and state departments of health.

The Program has been productive in this area as exemplified by studies that are resulting in an intervention strategy being developed for minimizing health effects associated with lead exposures in pregnant women and the unborn; developing innovative approaches for determining body burdens of persistent pollutants in populations who consume fish; and surveillance tools for evaluating reproductive health. All of the examples that follow demonstrate how the long-term, basic research supported by the SBRP, set in a use-oriented framework, contribute to societal needs.

4d.i Health Effects of Arsenic in People: Predicting Who is at Risk for Disease

In 1999, as part of a Columbia University Superfund Program led by Habibul Ahsan, a prospective cohort study of 12,000 men and women in Araihazar, Bangladesh was launched to investigate the health effects of arsenic (As) exposure, with an initial focus on skin lesions and skin cancers and also to establish a biorepository for future studies. The public health scientists, in collaboration with their earth science and geochemistry colleagues, first measured the arsenic concentration at 6,000 contiguous tube wells within a well-defined study area of approximately 26 square kilometers. This study, which involves people who have been drinking water with arsenic concentrations across an extraordinarily wide range (1-1000 ug/L), promises to be a landmark study in that it has the potential to definitively describe the dose-response relationships between arsenic in drinking water and health effects in humans. The study has already yielded some very important observations, and has created a repository of drinking water, blood and urine samples from all of the study participants.

For example, in order to identify clinically useful markers of As exposure or early toxicity, the Columbia team evaluated a series of growth factors (TGFα, VEGF and others) in urine from a subset of the study subjects and have found that urinary TGFα was strongly correlated with urinary As, suggesting that urinary TGFα is an early biomarker of chronic As exposure. This association was only found among subjects with skin lesions, not among normal subjects, suggesting that TGFα may mediate As-induced skin carcinogenesis. This finding is important. Just as we use the PSA test (prostate specific antigent) as a screening tool to monitor men for their risk for prostate cancer,

so too *the scientists believe that TGFα may be useful to screen arsenic-exposed populations for their risk of arsenic-induced cancer.*

This study has also found that certain genes play an important role in mediating the risk for arsenic-induced cancers. Some people have variants of genes that are involved in repairing DNA, the genetic material in cells that may predispose them to arsenic-induced disease. Still others are at risk because they have variants of other genes involved in sopping up active forms of oxygen. *As time goes on, this study in Bangladesh, which is also making heroic efforts to reduce the arsenic exposure of the population, will provide many findings that should guide United States policies concerning arsenic. Moreover, ongoing studies of pregnant women and young children will clearly delineate the health effects of exposure in these potentially vulnerable groups.*

[**Ahsan (Columbia University)**]

4d.ii Exposure Levels of Persistent Pollutants in Urban Anglers

One major accomplishment of the biomedical component of the Mount Sinai SBRP has been to define epidemiologic patterns of human exposure to persistent pollutants in the Hudson watershed. This work is based on the knowledge that a major route of human exposure to PCBs and other persistent pollutants is through consumption of contaminated fish, shellfish and crustaceans.

Sediments, fish, crustaceans and shellfish in the estuary of the lower Hudson River contain the residue of decades of contamination with persistent, bioaccumulative environmental pollutants. The complex mix of chemicals found in these waters includes PCBs, dioxins, the organochlorine pesticides chlordane and DDT, and mercury. Despite health advisories issued by the States of New York and New Jersey, many recreational and subsistence anglers still regularly consume their local catch and share it with family members and friends, and no data exists to document human exposure from eating fish or crabs caught in local waterways.

The goal of a project, conducted by Anne Golden and colleagues, is to determine through direct biological monitoring the levels of environmental contaminants that currently are present in the bodies of anglers and their family members. Golden hypothesizes that an increased consumption of locally caught fish and crabs will be associated with an increase in the body burden of organochlorides and heavy metals. Since the study began in 2001, the research team has collected data from 94 anglers representing three of the five boroughs of New York City. The population sampled includes a variety of racial groups. Minority anglers are believed to be especially reliant on local waters for food.

To assess human body burdens of persistent pollutants in persons who consume fish and crabs from the lower Hudson River watershed, the Mount Sinai scientists conducted a study of 46 local anglers in 1998-99. The questionnaire data showed that several of the aquatic species eaten most frequently – striped bass, bluefish, blackfish, and blue crabs – are among those listed in the advisories as most unsafe. The biomarker survey found that serum levels of total PCBs and highly chlorinated PCBs were higher in frequent consumers of locally caught fish and crabs than in infrequent consumers. The researchers observed similar patterns for the organochlorine pesticides chlordane and DDT, and for mercury.

The results of this pilot study indicate that anglers who eat fish and crabs from the lower Hudson River watershed have elevated body burdens of the same mix of persistent pollutants that are found in the sediments of these waterways. Further research is needed to examine geographic variation in body burden within various regions of the watershed and to elucidate disparities in body burden levels by socioeconomic status, race and ethnicity.

Golden believes that the data from this study, especially after additional analyses have been completed within the next year, will provide an extremely valuable input to decisions on whether to reopen the Hudson River commercial fishery.

[**Golden/Morland (Mount Sinai School of Medicine)**]

4d.iii Assessing Adverse Effects of Environmental Hazards on Reproductive Health in Human Populations

Reproductive disorders are an increasingly common problem within the United States. Many couples ready for parenthood and with seemingly good health have been disappointed to learn that they have reproductive difficulties. Decreases in human sperm concentrations and increases in infertility of young women have been reported over the past few decades, and are currently the focus of intense research and debate. Recent controversial evidence suggests that exposure to certain environmental chemicals may be contributing to the diminished reproductive capacity of adults who should be in their reproductive prime.

Adequate confirmation that reproduction is adversely affected by exposures to environmental chemicals can only be determined through methods that can evaluate both male and female fertility in toxicant exposed human populations. While many biomarker assays have been developed to assess reproductive health, most of them have focused on specific endpoints – such as infertility and early fetal loss – in women. Previous efforts have given little attention to evaluating general reproductive health in either of the sexes. *Bill Lasley leads a team of researchers at the University of*

California – Davis that has developed a novel biomarker assay that is capable of assessing the general reproductive health of all adults.

The enzyme-based immunoassay works by detecting the unique urinary metabolite of follicle-stimulating hormone (FSH), a gonadotropin that is involved in the regulation of the activity of the gonads in both men and women. Intact FSH is unstable in urine, and previous assays required the addition of a preservative to reduce the amount of dissociation of the two subunits that make up the heterodimer. This new method measures only the beta subunit unique to FSH and provides a measure of the total amount of FSH produced.

FSH is secreted by the anterior pituitary gland and controls a number of reproductive related functions. In men the hormone promotes the development of the tubules of the testes and the differentiation of sperm. In women FSH stimulates the development of follicles in the ovary and the biosynthesis of estrogen hormones.

As a result of the production and action of FSH in both sexes, levels of FSH metabolite excreted in urine provide information regarding both gonadal and pituitary function in all adults. High levels of the FSH metabolite indicate dysfunction at the gonadal level while lower than normal levels of FSH metabolite indicate diminished pituitary function. Self-collected, single-void urine samples can be used for this assessment, thus providing for a non-invasive assay that is expected to yield a high rate of compliance when used in epidemiological studies.

The development of this new bioassay is significant because it provides a general surveillance of reproductive health for both men and women. An additional benefit is that specific information can be obtained about the target of toxicity with respect to environmental exposures. Furthermore, this enzyme-based assay is relatively inexpensive and simple to carry out. Because of its simplicity, rapidity, and cost-effectiveness, this bioassay promises to be a useful screening tool of general reproductive health in epidemiological studies.

[**Lasley (University of California – Davis)**]

4d.iv Examination of Environmental Factors Leading to Childhood Leukemia

Childhood leukemia is the number one cause of cancer morbidity and mortality in children in the United States. The causes for 90% of childhood leukemias are unknown, and the environmental and genetic contributions to this disease are not well characterized. By studying the similarities in the pregnancies that harbored the initial genetic mutation in childhood acute myeloid leukemia, SBRP-funded scientists led by Patricia Buffler hope to identify dietary, environmental or other causes for the potentially harmful mutation. As part of the Northern California Childhood Leukemia Study,

Dr. Buffler and colleagues at the University of California-Berkeley examined 162 children newly diagnosed with childhood leukemia (age 0-14 years) and 162 matched control subjects randomly selected from the birth registry. Cases and controls were similar with respect to age, gender, race, Hispanicity, maternal education, and maternal age.

This study is unique in that it focuses on residential exposure rather than parental occupational exposure. The research team conducted in-home personal interviews with the primary care givers to collect detailed information on household pesticide usage including the name of each product; intended purpose (such as cockroach control); frequency of use; and time windows of use - 3 months prior to pregnancy, pregnancy, and the first, second, and third years of life.

The results of this study:

- Provide strong support of previous observations indicating that *exposure to household pesticides is associated with an increased risk of childhood leukemia.*
- Indicate that insecticide exposures early in life appear to be more significant than later exposures, with the h*ighest risk observed for exposure during pregnancy.*
- Distinguish between the risks associated with different types of pest control, *demonstrating a dose response relationship, and indicating the importance of the timing and location of exposure.*
- *Show a significant association between the use of professional pest control services and an increased risk of childhood leukemia.* The magnitude of this association was larger than what was seen for general insecticide or indoor pesticide exposures.

This information may enable us to educate the public concerning possible risks associated with exposures to pesticides, with particular emphasis on the timing and location of exposure.

[**Buffler (University of California – Berkeley)**]

4d.v The Harvard-Mexico Project on Maternal-Fetal Lead Exposure, Risks, and Prevention

Despite years of regulatory efforts and attention from public health authorities, lead remains a toxin of great concern. It is widespread in our nation's toxic waste sites, homes, and workplaces, with many opportunities for people to be exposed through water, food, and air. With respect to children, research shows lead has adverse effects on intelligence and other neurobehavioral attributes, even at very modest levels of exposure. Recent research has also demonstrated that these effects are not readily reversible, even with medical treatment, and are accompanied by effects that extend into adulthood, such as an increased risk of hypertension or kidney impairment.

A newly recognized but poorly understood aspect of lead toxicity stems from the fact that lead exposure results in large deposits of lead in bone (i.e., throughout the skeleton). These deposits persist for many years, even after exposure has ceased and blood lead levels have returned to normal.

During pregnancy, the physiologic changes that prompt a pregnant woman's bones to resorb (i.e., partially dissolve) in order to provide calcium to the growing fetal skeleton also release the lead stored in bone into a pregnant woman's circulation.

To address questions regarding the potential impact of maternal bone lead on the fetus and lactating infant, Superfund Basic Research investigators led by Howard Hu at the Harvard School of Public Health have partnered with investigators at the National Institute of Public Health in Mexico and created the Harvard-Mexico Project on Maternal-Fetal Lead Exposure, Risks, and Prevention (the "Harvard-Mexico Project"). Mexico City was chosen as the location for this research because its unique environmental lead exposure profile – leaded gasoline was phased out beginning in 1992 – left many women with low blood lead levels but high bone lead levels.

To conduct these studies, the project team developed and validated new analytical techniques including:

- K-x-ray fluorescence – a non-invasive and rapid method for measuring lead in bone
- A new method for estimating rates of bone resorption
- A new technique for measuring the ultra-low levels of lead that appear in blood plasma

The Harvard-Mexico Project has made a number of critical discoveries. Among these are:

- The bone lead levels of most women of reproductive age are easily measurable and are not low.
- These maternal bone lead levels are a major driving force of lead that appears in blood, plasma, and breast milk (luckily, despite this influence, most breast milk lead levels are low).
- Maternal bone lead levels can be used as biomarkers to predict lower newborn weight, head circumference, and body length.
- Maternal bone lead levels can be used as biomarkers to predict smaller weight gains from birth to one month of age, and lower measures of intelligence at age 2 years. This finding further confirms the importance of addressing lead exposure during pregnancy.
- Women who breast feed continue to have enhanced mobilization of lead from their bones into their blood, making potential lead toxicity an important issue to study in breast-feeding as well as pregnant women.

This SBRP-funded project has demonstrated that lead stores mobilized into the circulation of pregnant women pose a major threat to fetal development. Because bone lead stores, once accumulated, persist for decades, lead exposure can jeopardize the pregnancies of women even if their current lead exposures have subsided.

To address the public health challenge represented by the many thousands of women who have had lead exposure while growing up and who want to have healthy children, the Harvard-Mexico Project investigators have implemented a randomized trial of calcium supplementation as a low-cost strategy for reducing the toxic effects of maternal bone lead. Although the trial is still in progress, preliminary

studies provide evidence that calcium supplements reduce bone resorption rates during pregnancy as well as maternal blood lead levels during lactation.

Because there is no known method to reduce bone lead stores, the successful identification of a safe and easily implemented dietary intervention would constitute a major advance in our ability to minimize damage caused by lead. The ultimate significance of these studies will be the optimization of the care of pregnant women who have had exposure to lead and to minimize risk to their babies.

This research, which won a Progress and Achievement Award from the NIEHS in 1999, is a good example of a productive international research collaboration. Such collaborations will continue to become more important as globalization of industry and science continues to expand.

[**Hu (Harvard School of Public Health)**]

4d.vi Phosphate Treatment of Soil Reduces the Bioavailability of Lead in Humans

Hundreds of Superfund sites around the country are listed on the National Priority List because of contamination of their soils with lead (Pb). Soil can be accidentally ingested by small children through normal hand to mouth activity, and some of the Pb in the soil can subsequently pass into the bloodstream. It is now very well established that lead exposure in children is associated with cognitive deficits. Thus, Pb contaminated soil poses a real potential health risk to children.

Not all of the Pb in ingested soil actually enters the bloodstream; some actually passes through the entire length of the gut unabsorbed. The fraction that is absorbed is referred to as the bioavailable fraction. Clearly, if one could develop technologies or soil treatments that would reduce the fraction of Pb that is absorbed into the blood, the cleanup level for Pb in soil would increase. In the extreme example, if an *in situ* soil treatment could reduce Pb bioavailability to 0%, then we would not have to haul and dispose Pb-contaminated soils at all.

Phosphate, as found in typical agricultural phosphate fertilizers, can form insoluble complexes with Pb. For this reason, USEPA scientists have been interested in evaluating the effect of phosphate treatment, i.e., the application of standard phosphate fertilizer, on Pb bioavailability. Some work in experimental animals suggested that phosphate treatment might be effective in reducing Pb absorption, but the extrapolation of those findings to human populations was problematic.

Joseph Graziano leads a team of scientists at Columbia University. Using a sophisticated technology known as stable isotope dilution, they have been able to administer minute amounts of Pb-contaminated soils (in small gelatin capsules) – some phosphate treated, and some not – to normal human volunteers in an effort to estimate the effect of phosphate treatment on Pb bioavailability. *Their results are very exciting in that phosphate treatment appears to dramatically*

reduce the absorption of soil Pb, indicating that the addition of phosphate fertilizer to contaminated soil is a viable approach to remediation at many Superfund sites. For example, Pb absorption from a contaminated soil obtained from a former smelter site in Joplin, Missouri, averaged 36% among a group of adult volunteers. However, Pb absorption from an adjacent soil that had been "fertilized" in the field 18 months earlier averaged only 15%. *This 57% reduction in Pb bioavailability implies that the Pb hazard at many Superfund sites can be effectively eliminated in a very cost effective manner.* The investigators at Columbia University are about to meet with local USEPA officials in Joplin, Missouri and Tar Creek, Oklahoma to discuss the obvious implications of their findings.

[**Graziano (Columbia University)**]

4d.vii Environmental and Biochemical Risk Factors for Parkinson's Disease

Parkinson's disease (PD) is a chronic neurodegenerative disease that affects approximately one million people in the United States population, primarily at ages 50 years and older. The clinical hallmarks of PD are abnormally slowed movement, muscle rigidity, tremor of muscles at rest, and difficulties walking and standing. Among the neurodegenerative diseases, PD is second in frequency to Alzheimer's disease. The underlying physical basis of PD is a loss of brain cells that produce dopamine, the neurotransmitter required for coordinated muscle movements. Only about 10% of PD cases can be attributed to direct (Mendelian) inheritance, whereas causal factors are essentially unknown for the vast majority of PD cases. Evidence has grown during the past 20 years that environmental factors may play significant roles in PD etiology.

Recent thinking emphasizes the importance of gene/environment interactions, whereby persons with genetically-determined susceptibility traits are at greatest risk of PD induced by environmental chemicals. The chemicals of most interest include pesticides, metals, and industrial solvents. Somewhat paradoxically, cigarette smokers have consistently been found to have lower PD risks than non-smokers, suggesting a possible "neuroprotection" afforded by nicotine or other components of cigarette smoke. Caffeine has also been proposed as a neuroprotective agent, based on some epidemiologic research. In view of this background, and the likelihood that chemical exposures that commonly occur at hazardous waste sites may be involved in PD pathogenesis, there is ample justification for rigorous epidemiologic research that combines the tools of epidemiologic study design, environmental exposure assessment, biochemical toxicology, and molecular genetics.

Harvey Checkoway at the University of Washington is leading an SBRP-funded project to examine the contributions of environmental factors and genetic susceptibility traits to the causation of PD. The environmental agents of greatest interest are those that have the potential to damage (or protect) neurons that produce the neurotransmitter dopamine. In addition Checkoway's group is investigating

variant forms of genes that may render persons especially sensitive to the effects of environmental chemicals.

The research team observed the predictable inverse relation between cigarette smoking and PD risk. Several recent studies suggest potentially protective effects of caffeine, but this study found no association with either coffee consumption or total caffeine intake. They did, however, detect a reduced PD risk among persons who consume two or more cups of tea per day – this finding was not anticipated, but deserves further investigation as tea contains some anti-oxidants chemicals.

Analyses of exposures to pesticides indicate possible associations with some types of pesticides from occupational sources, although the researchers have not seen evidence for associations of PD with home use of pesticides. Further analyses of pesticides and other environmental agents, including metals and solvents, are underway.

Checkoway's investigation of genetic susceptibility traits has been extensive, including analyses of multiple gene variants. The most notable result thus far has been an association with a form of the monoamine oxidase B (*MAO-B*) gene. The enzyme encoded by this gene is especially relevant to PD because it catabolizes dopamine, and activates some neurotoxic chemicals that may be involved in PD induction. Checkoway's group has previously shown that the seemingly protective effect of cigarette smoking was only found among persons with a particular variant of a non-coding (intronic) region of the *MAO-B* gene, the G allele. The researchers have their analysis of the data, and observed that this gene/environment interaction was confined to men; women smokers had a reduced PD risk irrespective of *MAO-B* gene type. One possible implication of this finding is that estrogen may play a complex interactive role with smoking and genotype. The research team is continuing its exploration for mutant forms of *MAO-B* in the coding and promoter regions, which in theory should have stronger functional consequences, than intronic variants, on enzyme function. Other ongoing work involves identification of mutations of the M2 class of the glutathione S-transferase (*GSTM2*) gene. There is good evidence that the *GSTM2* enzyme inactivates toxic metabolites of dopamine, and may therefore be pertinent to PD pathogenesis. The search for novel mutations in the *MAO-B* and *GSTM2* genes will be accompanied by in vitro studies of their enzymatic functional significance.

Future work will focus on characterizing interactions between genetic polymorphisms and environmental exposures (gene/environment interactions), as well as interactions among genes (gene/gene interactions). *This research may ultimately provide valuable new information that can lead to PD prevention strategies, and may serve as a template for investigating the interplay of genes and environment on risk for chronic neurological diseases.*

[**Checkoway (University of Washington)**]

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4e. Contributions to Understanding Mechanisms of Toxicity of Superfund Chemicals

Understanding the mechanisms whereby toxicants induce adverse human health effects is at the heart of the Program. Since the inception of the SBRP, we have supported many different lines of investigation that are elucidating, at a molecular level, how toxicants promote disease processes. The data generated from these studies are critical to the Superfund efforts and serve as the underpinning of future public health intervention strategies.

Investments that the SBRP made 10 to 15 years ago are now being realized. For example, our long-term support of research on benzene toxicity has led to the development of chromosomal markers that may provide important molecular clues for childhood leukemia. Investments we make today have future potential payoffs with a multitude of applications.

The following eight summaries exemplify the many stages of mechanistic research – from laboratory-based studies unraveling disease pathways, to human-based mechanistic studies – that are providing practical information to inform the risk assessment process.

4e.i Unraveling the Origins of Childhood Leukemias

The study of childhood leukemia is important because leukemia is the number one cause of cancer morbidity and mortality in children in the United States. The causes for 90% of childhood leukemias are unknown and the environmental and genetic contributions to this disease are not well characterized. As part of ongoing SBRP-funded research, scientists led by Drs. Martyn Smith and Joseph Wiemels are using a sophisticated molecular technology to help unravel the origins of the mutations that lead to some childhood leukemias. Leukemia may arise following the abnormal fusion of two genes, which results in production of abnormal proteins. The research team studied leukemias stemming from the fusion of genes known as AML1 and ETO. This fusion, referred to as t(8;21)AML1-ETO translocation, is present in a type of childhood acute myeloid leukemia (AML), the most difficult type of leukemia to treat. The AML1 protein is a "master" regulator of gene activity for blood cell development from stem cells. If the AML1-ETO fusion occurs in just one out of the thousands of stem cells a person is born with, then the stage is set for a second gene mutation to trigger the leukemia, even if the second mutation occurs years later.

The research team examined both diagnostic blood samples and neonatal "heel prick" blood spots from children with AML, two of whom were older than 10 years of age at diagnosis. The scientist's detected t(8;21)AML1-ETO in the "heel prick" blood samples for 5 individuals, showing that the translocation must have occurred *in utero*, *providing unambiguous evidence of prenatal origin in these*

cases and suggesting that exposures of the fetus before birth could be critical in producing childhood leukemia.

The discovery of this "latency period" between the initiating genetic event and full development of leukemia suggests that even some adult leukemia may start in the womb. These results further implicate the fetal period as a distinctly susceptible period for chromosomal translocation, perhaps the usual period for such events among pediatric leukemias.

In addition to the current report of the *in utero* t(8;21)AML1-ETO translocation in leukemia patients, the research team has confirmed that two other mutations, the MLL and TEL-AML1 translocations, have prenatal origins. Childhood cancers besides leukemia, particularly sarcomas, are commonly associated with translocations. *The t(8;21)AML1-ETO translocation biomarker has particular significance in that it is not merely associated with acute myeloid leukemia, but is on the causal pathway of the environmentally-induced cancer and as a result should be a better predictor of increased cancer risk than existing biomarkers.*

[**Smith (University of California – Berkeley)**]

4e.ii Human Dosimetry for Assessment of Exposure to Volatile Compounds

SBRP-funded researchers at the University of Washington, led by Michael Morgan are investigating the pharmacokinetics of volatile organic solvents. Using human volunteers given controlled exposure by inhalation, they are working to characterize the uptake, distribution, metabolism, and excretion of a compound and its biotransformation products in individuals under conditions of exposure that approximate those expected at hazardous waste sites.

Morgan has placed major emphasis on exploring the differences among normal individuals in pharmacokinetics, and in determining the underlying explanations for those differences. During the course of the project, over 200 controlled exposures have been conducted, using aromatic and aliphatic solvents given singly or in pairs. Each exposure was of two hours' duration, during which the subject was at rest or was exercising at a moderate rate to simulate, respectively, residence near or work at a waste site. For safety reasons, the exposure concentration was at or below the level permitted or recommended by government or expert agencies. For most of the solvents studied, the researchers observed a wide variation in pharmacokinetics; after identical exposure conditions, levels in body fluids ranged by nearly ten-fold.

Simultaneously with the experimental work, Morgan's group developed and refined a detailed physiologically-based mathematical model of the pharmacokinetic processes in order to describe the individual results. The model includes five to seven compartments that describe the response of

different tissues in the body. Each compartment has properties that can be quantitatively adjusted to match individual characteristics, such as body mass, fat percentage, and resting pulmonary ventilation rate. The researchers have tested the model for its ability to describe accurately the time course of solvent concentrations in exhaled breath, venous blood, and urine of each exposed subject. To achieve the highest accuracy, it was necessary to determine the solubility of the exposure compound in blood drawn from the subject prior to exposure because of considerable variation among individuals in this physico-chemical parameter. In addition, the model is sensitive to the distribution of blood flow to the adipose and liver tissue compartments. Since this information cannot be obtained for individuals, the distribution to these compartments is adjusted to provide the best fit of the model to the data. For three compounds studied extensively to date, the adjusted model is able to account for between 85% and 95% of the variation among individuals. The major characteristics that determine individual variation in response to exposure are age, body mass, fat percentage, and to a lesser extent, gender. Probability distribution functions for the fitted parameters have been developed as a result of the model-fitting procedure and these will permit accurate simulation of the response of most individuals to actual exposures.

One significant consequence of this work is improved ability to interpret biomarker data taken from exposed individuals to deduce the extent of exposure when it is unknown. For example, a hazardous waste site worker could provide a urine sample at the end of a shift, or at some convenient time after the end of a shift involving an unknown exposure. Using the personal characteristics identified earlier, the level of urinary metabolites and the parent compound can be entered into the mathematical model, and the result will be an estimate, with uncertainty, of the dose and exposure concentration of the solvent during the previous shift. Therefore, the details of the relationship between exposure and the presence of exposure markers in biological samples at later times are now becoming available. The ability to account for almost all of the factors causing inter-individual differences in pharmacokinetics will permit reduction in the uncertainty that now hampers quantitative risk analysis and subsequent risk management practices. While further demonstration is needed, the approach developed in this research may soon have value in public health practice, as human biomonitoring activity increases.

[**Morgan (University of Washington)**]

4e.iii Biomarkers of Neurotoxicant Exposure and Neurodegeneration

SBRP-funded scientists at the Oregon Health & Science University (OHSU), led by Mohammed Sabri and Peter Spencer have made a major advance in identification of organic solvents with the potential to damage the nervous system. They have determined the relative toxic potencies of organic solvents and the biochemical mechanisms by which they effect their toxicity.

Repeated exposure to mixtures of organic solvents has caused epidemics of extremity muscle weakness and altered sensation (peripheral neuropathy) among workers in North America and Japan (1970s), parts of Europe (1980s) and China (today). Such exposures continue to pose a health threat at Superfund sites. Most outbreaks have been associated with solvent mixtures, which include chemicals with straight hydrocarbon chains (aliphatic) and those with ring structures (aromatic). Previously it was thought that only certain *aliphatic* compounds, such as n-hexane, had the potential to induce peripheral neuropathy. n-Hexane is activated in the body to 2,5-hexanedione (2,5-HD) – a gamma-diketone that polymerizes proteins and induces nerve fiber degeneration. *Aromatic* compounds were considered to be relatively safe because they were thought to be unable to form gamma-diketones. The OHSU research has proved this long-held assumption to be incorrect.

Sabri and Spencer have shown that 1,2-diacetylbenzene (1,2-DAB), an *aromatic* derivative of the solvent 1,2-diethylbenzene, behaves like a gamma-diketone of much greater potency than its *aliphatic* analogue. Rats and mice treated with 1,2-DAB develop limb weakness more rapidly than those treated with much lower doses of 2,5-HD. Both neurotoxic compounds, but not their non-neurotoxic analogues (1,3-DAB and 2,4-HD), form colored pigments when they react with proteins, thereby providing a potential biomarker of exposure. Targeted proteins have a high content of the amino acid lysine, a portion of which is targeted by the neurotoxic chemicals. Neurofilament proteins, which are synthesized in the body of the nerve cell (neuron) and then transported along the process (axon) that connects the neuron with muscle and skin, are prime targets for polymer formation and transport failure resulting in neurofilament accumulation in large axonal swellings. Whereas the potent aromatic gamma-diketone 1,2-DAB reacts with proteins rapidly and thereby arrests neurofilament transport proximally in the spinal cord and spinal roots, the less potent aliphatic compound reacts slowly and causes axonal swellings in peripheral nerves supplying the limbs. The attendant neurobehavioral deficits reflect the distinct sites of neuropathology.

This work has opened up a new line of investigation into the neurotoxic properties of aromatic organic solvents. *The key observation is the demonstration that neurotoxic solvent chemicals form colored pigments with proteins (chromogenicity) and non-neurotoxic solvent chemicals lack this chromogenic property.* The interdependence of chromogenicity and neurotoxicity has called into question whether a number of other reportedly chromogenic aromatic solvents possess neurotoxic potential. Because many of these are in widespread commercial use, study of this question will be highly relevant to risk

assessment, decision-making processes at Superfund sites and to public health. However, Sabri and Spencer have been able to reduce uncertainty in the risk assessment process by demonstrating that organic solvents lacking the chromogenic property also lack the ability to induce neurodegeneration. Finally, the chromogenic property of the neurotoxic substances opens the possibility of creating a biomarker of exposure to neurotoxic organic solvents.

This research provides new tools to understand the relationship between the structure of environmental pollutants and their potential to damage human health. In particular, scientists are now able to compare and contrast the actions of neurotoxic substances with high-potency (1,2-DAB) and low-potency (2,5-HD) with structural homologues (1,3-DAB, 2,4-HD) that lack comparable toxicity. These compounds are now being used to isolate the neurotoxic property of solvent diketones through gene- and protein-expression profiling. By subtracting the effects of the non-neurotoxic diketones from those associated with the neurotoxic substances, it should be possible to isolate genomic and proteomic responses that discretely correspond to neurotoxicity. *These fingerprints of neurotoxicity should be useful for the rapid screening of other compounds for neurotoxic potential.*

[**Sabri/Spencer (Oregon Health & Science University)**]

4e.iv Genotoxic Mechanisms of Arsenic in Mammalian Cells

Epidemiological data gathered for more than a century have shown that arsenic is a potent human carcinogen. However, the mechanism(s) by which arsenic induces cancer is not known. Earlier studies of arsenic have reported that arsenic does not cause mutations in genes, but does cause large changes in chromosome structure. The failure of arsenic to induce gene mutations in mammalian cells has been taken as evidence that arsenic is a non-genotoxic carcinogen.

Using a state of the art mutagenic assay system based on human-hamster hybrid (AL) cells, s*cientists at Columbia University led by Tom Hei have shown that arsenic is indeed mutagenic to endogenous genes in mammalian cells and that it induces mostly large multigene deletions. This arsenic-induced effect is mediated through the production of highly reactive forms of oxygen known as hydroxyl radicals. Moreover, they have demonstrated that both the nucleus and mitochondria of cells are adversely affected by these activated forms of oxygen.*

These findings have important therapeutic implications for individuals who have been exposed to arsenic. Indeed, millions of people in the United States and around the world are exposed to unacceptable levels of arsenic in drinking water. The new findings add to the growing body of scientific evidence that the so-called anti-oxidant vitamins, such as vitamins E, C and carotenoids, might have therapeutic utility in preventing the adverse effects of arsenic. Indeed, another study by Columbia University scientists, being carried out in a region of Bangladesh that has very high

drinking water arsenic levels, is testing the efficacy of anti-oxidant vitamins in reducing the incidence and severity of skin lesions.

[**Hei (Columbia University)**]

4e.v The Role that Arsenic Metabolism Plays in its Toxicity

Arsenic in humans causes cancer of the skin and internal organs. Some of the ways the American public is exposed to arsenic is in their drinking water, food, contact with chromated-copper-arsenic (CCA) impregnated wood used in children's playgrounds and residential decks, and in Ironite© fertilizer. Investigators under the direction of Vas Aposhian (University of Arizona) use human tissues to study how arsenic is processed by the human body. The major thrust of these efforts has been to purify and understand the catalysts (enzymes) in the human body that control how the human body processes arsenic. This processing or biotransformation was once thought to be a means of detoxifying arsenic, but new work in Aposhian's laboratory involving the isolation, the structure determination, and the characterization of the properties of these catalysts clearly demonstrates that these biotransformation processes can make arsenic more toxic in the body. They have shown that the human body converts arsenic to more toxic forms, which stick to body constituents very tightly, stay in the body longer, and are more harmful. *This research team was the first to identify two new more toxic forms of arsenic in human tissues and urine (MMA^{III} and DMA^{III}) – these forms are speculated to be the cause of arsenic-related cancers.*

Most recently, Aposhian's laboratory identified a number of new ways that humans detoxify these most toxic forms of arsenic. It appears that there is more than one mechanism by which the human detoxifies arsenic. In addition, there is a difference in how and to what extent people process arsenic. There is an unusually high variation in what forms of arsenic they make in the liver and excrete in the urine. The researchers hypothesize that this is due to changes in the genes that make the enzymes that biotransform arsenic and they will soon test this hypothesis by isolating the DNA from blood of humans exposed to arsenic in their drinking water. Aposhian's team now has a method for detecting the change of a single letter in the genetic code that tells the human body how to process various forms of arsenic. All of this information, including Aposhian's demonstration that the arsenic of CCA is absorbed when given by mouth, is expected to be of help to people determining how arsenic causes cancer and to improve risk assessments for arsenic and humans.

[**Aposhian (University of Arizona)**]

4e.vi Superfund Chemicals, Nutrition, and Endothelial Cell Dysfunction

The central hypothesis of Bernard Hennig's (University of Kentucky) research is that specific PCBs can increase endothelial cell dysfunction and atherosclerosis by oxidant mediated mechanisms in the presence of specific lipids, such as lipids rich in n-6 unsaturated fatty acids. Hennig also hypothesizes that nutrients or compounds/chemicals with antioxidant properties will provide protection against PCB/lipid-mediated endothelial cell dysfunction.

Hennig's laboratory group previously determined that PCB 77, an aryl hydrocarbon (Ah) receptor agonist, can cause disruption of endothelial barrier function. This was supported by an increase in oxidative stress as measured by enhanced 2',7'-dichlorofluorescein (DCF) fluorescence and activation of the oxidative stress-sensitive transcription factor NF-kB. Subsequently, they tested the protective effects of antioxidants, e.g., vitamin E (a-tocopherol), on endothelial cell activation induced by PCB 77. Vitamin E completely blocked PCB 77-mediated endothelial barrier dysfunction. This protective effect by vitamin E was associated with a decrease in both oxidative stress, as well as in NF-kB activation.

Hennig and his research group expanded their studies with other coplanar PCBs to provide evidence that an intact AhR receptor is critical during oxidative stress-mediated endothelial inflammatory events that are initiated by exposure to PCBs. Endothelial cells were exposed to PCB 153 and to three coplanar PCBs (PCB 77, PCB 126, or PCB 169). In contrast to PCB 153, which is not a ligand for the Ah receptor (AhR), all coplanar PCBs disrupted endothelial barrier function. All coplanar PCBs increased expression of the CYP1A1 gene, oxidative stress (DCF fluorescence) and the DNA binding activity of NF-kB. The increase in NF-kB-dependent transcriptional activity was confirmed in endothelial cells by a luciferase reporter gene assay. In contrast to PCB 153, coplanar PCBs that are AhR ligands increased endothelial production of interleukin-6 (IL-6) and the expression of the adhesion molecule VCAM-1. This research provides *in vivo* evidence suggesting that binding to the AhR is critical for the proinflammatory properties of PCBs. Twenty hours after a single administration of PCB 77, VCAM-1 expression was increased only in wild-type mice, while mice lacking the AhR gene showed no increased staining for VCAM-1. *These data provide evidence that coplanar PCBs, agonists for the AhR, and inducers of cytochrome P450 1A1, produce oxidative stress and an inflammatory response in vascular endothelial cells.* An intact AhR may be necessary for the observed PCB-induced responses. *These findings suggest that activation of the AhR can be an underlying mechanism of atherosclerosis mediated by certain environmental contaminants.*

Hennig's team continued studies in collaboration with Dr. Bruce Hammock's laboratory at UC-Davis investigating the mechanisms of the cytotoxic interaction of the dietary lipid, linoleic acid, and its metabolites with PCBs. Selected dietary lipids may increase the atherogenic effects of environmental chemicals, such as PCBs, by cross-amplifying mechanisms leading to dysfunction

of the vascular endothelium. They hypothesize that the amplified toxicity of linoleic acid and PCBs to endothelial cells could be mediated in part by cytotoxic epoxide metabolites of linoleic acid called leukotoxins (LTX) or their diol derivatives (LTXD). Pretreatment with the cytosolic epoxide hydrolase inhibitor 1-cyclohexyl-3-dodecyl-urea partially protected against the observed LTX-induced endothelial dysfunction. Endothelial cell activation mediated by LTX and/or LTXD also enhanced nuclear translocation of the transcription factor NF-kB and gene expression of the inflammatory cytokine IL-6. Inhibiting cytosolic epoxide hydrolase decreased the LTX-mediated induction of both NF-kB and the IL-6 gene. Most importantly, inhibition of cytosolic epoxide hydrolase blocked both linoleic acid-induced cytotoxicity, as well as the additive toxicity of linoleic acid plus PCB 77 to endothelial cells. *These data suggest that cytotoxic epoxide metabolites of linoleic acid play a critical role in linoleic acid-induced endothelial cell dysfunction.* Furthermore, the severe toxicity of PCBs in the presence of linoleic acid may be due in part to the generation of epoxide and diol metabolites. *These findings have implications in understanding interactive mechanisms of how dietary fats can modulate dysfunction of the vascular endothelium mediated by certain environmental contaminants.*

[**Hennig (University of Kentucky)**]

4e.vii Explanation of the Differences in Dioxin Sensitivity and Resistance

Chlorinated dioxins and dioxin-like chemicals, including certain PCBs, are globally distributed environmental contaminants that occur at especially high concentrations at many Superfund sites. These chemicals are highly toxic to developing embryos. There are well-known differences among species and individuals in the sensitivity to the developmental effects of these chemicals, but the mechanisms underlying these differences are not well understood. In addition, little is known about the effects of long-term exposure to high levels of dioxins and PCBs, such as occurs at some Superfund sites.

There is increasing evidence that tolerance or resistance to dioxin-like chemicals can develop after long-term exposure of animal populations to these contaminants. Mark Hahn and his research team in the Boston University SBRP program are studying a population of estuarine fish, Atlantic killifish (*Fundulus heteroclitus*), inhabiting New Bedford Harbor, MA (NBH), a Superfund site that is highly contaminated with PCBs and other chemicals. Their research, and that of investigators at the USEPA laboratory in Narragansett, RI, has shown that NBH killifish are much less sensitive to effects of PCBs and dioxins as compared to killifish from less contaminated sites such as Scorton Creek, in Sandwich, MA (SC). This resistance is passed on from parents to their offspring, suggesting that it is a genetic trait that has evolved in the NBH fish population.

The objective of this research is to identify the cause of dioxin/PCB resistance in NBH killifish. In fish, as in mammals, the effects of dioxin-like chemicals occur through their interaction with a protein known as the aryl hydrocarbon receptor (AHR). Hahn's group has identified two distinct aryl hydrocarbon receptors (AHR1 and AHR2) in killifish and other species of fish, and is investigating the role of these two receptors in the dioxin/PCB resistance that has evolved in NBH fish.

Two recent findings provide insight into how NBH fish might resist dioxin effects:

- The researchers have identified and isolated the gene coding for a protein that is able to inhibit the ability of dioxins and PCBs to cause effects through their interaction with the AHR1 and AHR2 proteins. The levels of this inhibitory protein, known as AHR repressor (AHRR), can be increased by treatment of fish with dioxins or PCBs. Preliminary studies show that the amount of AHRR is not increased in adult fish from NBH. However, this protein could be active in NBH fish embryos and contribute to their resistance to dioxins and PCBs; this possibility is currently under investigation.
- A second possible resistance mechanism involves polymorphisms (slight variations) in genes coding for the AHR1 or AHR2 proteins. It appears that the AHR1 gene in killifish is highly variable among individuals. NBH fish (dioxin resistant) and SC fish (dioxin sensitive) differ in the types of AHR1 variants they possess, suggesting that certain variants confer resistance to fish possessing them.

The degree of risk that dioxins and PCBs pose to the health of humans and the environment is controversial. The key to resolving uncertainties concerning this risk is to determine dioxin's mechanism of action and to identify the factors that influence the sensitivity or resistance of individuals or populations. *Understanding how dioxin/PCB resistance occurs in these highly exposed fish will improve our ability to predict the sensitivity of humans and wildlife to these compounds.* In addition, this research will help us understand the long-term impact of chemicals at this and other Superfund sites. For example, following the persistence or loss of resistance over time will help to evaluate the success of remediation at these sites.

The results of this research have been communicated to the scientific community through publication in peer-reviewed journals. In addition, these findings have been more broadly disseminated through local and national coverage on National Public Radio's *Living on Earth*, and through invited presentations to state and federal risk assessors.

[**Hahn** (**Boston University**)]

4e.viii Genotoxicity of Metals in Complex Mixtures

SBRP investigators at the University of Cincinnati are studying the mechanism of action of two cancer-causing metals (chromium and arsenic), which are often found in complex mixtures with organic contaminants at Superfund sites. Exposure of humans to certain forms of chromium (chromate) and arsenic (arsenite) has been shown to increase cancer risk. However, the mechanism by which these metals act is not been well understood. In addition, increased cancer risk appears to be even greater in individuals exposed at the same time to other cancer-causing chemicals (e.g., cigarette smoke or other organic combustion products such as polycyclic aromatic hydrocarbons [PAHs]). Therefore, it is important to understand how mixtures of metals and PAHs act synergistically to cause cancer. SBRP investigators led by Kathleen Dixon are focusing on the ability of these metals alone and in mixtures to cause genetic damage (genotoxicity), which is a precursor to cancer development.

Exposure of humans to chromate as a dust or aerosol increases the risk of lung cancer, and also appears to be associated in an increased risk of kidney and bladder cancer. Dixon's group has shown that introduction of chromate into the lungs of mice causes the induction of genetic changes (mutations) in lung tissue; smaller increases in mutations are observed in liver and kidney tissue. These increases in mutations correlate with the accumulation of chromate in respective mouse tissues. Since mutations are an underlying cause of cancer, *these results suggest that the carcinogenic activity of chromate is due to its ability to cause mutations. Interestingly, the ability of chromate to cause mutations in mouse tissue could be modulated by altering the tissue levels of the cellular anti-oxidant, glutathione.* This result is consistent with other work that shows that glutathione participates in chromate metabolism and that chromate causes oxidative-type damage to DNA. Further studies will be required to determine whether manipulation of natural anti-oxidants might serve to protect against the carcinogenic effects of chromate.

Exposure of humans to arsenic in drinking water is associated with the development of a variety of cancers, especially cancers of the skin, lung, and liver. Unlike chromate, arsenite by itself at low doses does not appear to be genotoxic. However, arsenite exposure appears to increase the mutagenic activity of other carcinogens. SBRP investigators have shown that when cultured mouse liver cells are exposed to a mixture of arsenite and benzo[a]pyrene (B[a]P), a carcinogenic PAH, there is a dramatic increase in mutagenesis compared with arsenite or B[a]P alone; arsenite by itself did not seem to cause any significant increase in mutagenesis. The increase in mutagenesis observed with B[a]P and arsenite correlate with an increased accumulation of DNA adducts (DNA damage that serves as a precursor to mutations).

Preliminary data suggest that arsenite may cause an increase in DNA adducts, and thus mutations, by two mechanisms. First, SBRP investigators have shown that *arsenite alters the pattern of expression of*

genes required for B[a]P metabolism and detoxication which could lead to the observed increase in B[a]P DNA adducts. Second, these investigators have shown that *arsenite amplifies and prolongs cell signaling responses to DNA damage*, suggesting that the DNA damage persists longer, possibly due to a DNA repair defect. Experiments were conducted to assess the ability of arsenite to cause increases in DNA adducts and an enhancement of mutagenesis in B[a]P-treated mice. If these animal experiments confirm the cell culture results, intervention studies will be carried out in the mouse to test possible methods for reducing the co-mutagenic activity of arsenite.

These studies are the result of collaborative interaction among University of Cincinnati SBRP investigators. Their significance lies in the elucidation of mechanisms by which metals acting alone and in mixtures with organic contaminants cause genotoxic damage, and thus increase cancer risk. Further development of these data could contribute to the development of quantitative risk assessment methods for mixtures that more accurately estimate human health risk under environmental exposure conditions.

[**Dixon (University of Cincinnati)**]

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4f. Contributions to the Development and Advancement of Remediation Processes

The SBRP has a unique role at NIEHS as we fund research that goes beyond the biomedical arena. In particular, we support the application of engineering sciences as a primary prevention strategy to improve public health. Our principal focus in this area is in the remediation of hazardous waste sites.

SBRP has supported basic research to understand the principles and processes necessary to clean up persistent toxics in groundwater, sediments and soil. These are all areas of high priority to the Superfund program. Innovative remediation processes developed by the Program have the potential to improve removal, sequestration or destruction of previously untreatable contaminants; provide more cost-effective methods; or offer integrated contaminant detection and remediation processes.

This has been a very productive component of the program as evidenced by the examples below, the number of patents, SBIR/STTR awards and other applications of these technologies (see Section1 D). However, one of the biggest challenges in this area continues to be the translation of basic studies into application. We have addressed this challenge aggressively through our many information and technology transfer efforts, as discussed in Section 2. Of particular importance to this process is our close collaboration with the USEPA. By working closely with the USEPA headquarters and regional staff we continue to identify additional opportunities for application of the Program's advances.

4f.i Technology Transfer of Steam Injection for Soil and Groundwater Cleanup

Drs. Kent Udell and James Hunt at the University of California-Berkeley showed that liquid solvents trapped in soils are able to move towards groundwater supplies via a process called dense vapor migration. They also demonstrated that steam injection effectively removes these trapped solvents from soils at hazardous waste sites. SBRP support of the fundamental science of thermal remediation at the University of California-Berkeley has generated a new national and international industry, and a solid technical basis for USEPA policy on source removal for soil and groundwater cleanup. It is now clear that many sites previously considered as untreatable can be remediated with reasonable cost and acceptable certainty using thermal remediation techniques. Thermal techniques have been used by several industries and in many countries to provide a permanent solution to problems that would otherwise persist for centuries. Prior arguments that there is not technology capable of removing heavier than water liquids from the subsurface have been negated, giving fresh impetus to the push for aggressive environmental cleanup by regulatory agencies.

The Berkeley research led to a very successful full-scale demonstration of Steam Enhanced Extraction at Lawrence Livermore National Laboratory, and then to a showcase industrial application at the Visalia Pole Yard in Visalia, California – one of the first sites placed on the National Priority List. ***This work is considered to be a break-through application of remediation technology, thus earning the first and only USEPA Remediation Technology Development Award*** for "technical excellence in the development of *in situ* thermal treatment technologies". The application of Steam Enhanced Extraction at that site decreased the financial liability of the site by $85 million for a cost of about $15 million, demonstrating a clear financial incentive for publicly owned corporations to take a more aggressive approach to environmental restoration.

The transfer of the SBRP-supported thermal technology continues with growing applications at Superfund sites and other sites of national interest. Specifically, the use of Steam Enhanced Extraction is:

- Included as the selected cleanup remedy in the record of decision for the Wyckhoff Superfund site adjacent to Eagle Harbor near Seattle. A pilot test of the technology, consisting of about 10 injection and 10 extraction wells is now underway.
- Being considered for the remediation of the Baxter and McCormick Superfund site near Stockton, California with a full-scale design completed in sufficient detail for costing purposes. The project is now under funding consideration by the USEPA. This site is one of the largest wood treating chemical spills in the country and defies all other techniques for remediation in any reasonable time frame.

Steam injection was also applied at Cape Canaveral at Launch Complex 34. This site is particularly important in that the steam technology is the third technique to be demonstrated in adjacent plots. The use of steam injection at that site is also unique in its application through the co-injection of air with the steam. This approach, first considered by researchers at the University of Stuttgart in Germany for the removal of semi-volatile non-aqueous phase liquids (NAPL) in the vadose zone, was extended for application to volatile NAPL found in the saturated zone through NIEHS funding. That research developed the theoretical underpinning of the value of air co-injection with the steam in the elimination of accumulation of the NAPL at the steam condensation front. Such accumulation has been shown to be the requisite step leading to uncontrolled downward migration of the non-aqueous phase liquid during thermally enhanced cleanup operations. In all cases, including controlled two dimensional laboratory experiments, the simultaneous injection of air and steam increases the NAPL compound removal rate beyond that which could be achieved by either pure steam injection or pure air sparging.

Of near equal importance is a project initiated at Loring Air Force Base in Maine where steam is to be injected into fractured rock, which is contaminated by NAPL trapped in the fractures. This is a large project where both the State of Maine and the USEPA are actively engaged in the application and

evaluation of the potential of Steam Enhanced Extraction to remediate fractured geologies. SBRP contributes to that project through the transfer of the steam injection technology expertise to the multi-agency consortium of stakeholders to ensure that the most current engineering knowledge is applied to the contaminants and media of interest at that site.

International application has also been gaining momentum. Expertise from SBRP-funded work has been applied to a PCE- and kerosene-contaminated site in Denmark with success, and Steam Enhanced Extraction is being used routinely in the Czech Republic, including the first successful remediation of a fractured bedrock site. Also of interest is the successful pilot demonstration of the technology at the Pancevo site in the former Yugoslavia, now Serbia, which experienced a catastrophic release of millions of pounds of the highly toxic chemical, 1,2 DCA due to the NATO bombings in 1999.

[**Udell** (**University of California – Berkeley**)]

4f.ii Phytoremediation of Toxic Wastes

Organic solvents, such as the dry cleaning solvent trichloroethylene (TCE), are toxic, chemically unreactive, heavy, insoluble in water, and tend to rapidly move through the soil and into ground water, contaminating drinking water reservoirs. Conventional remediation technologies for these compounds are expensive and time consuming, and may result in the formation of toxic by-products.

Following more than a decade of basic, mechanistic research, Milton Gordon and Lee Newman at the University of Washington SBRP have developed and implemented phytoremediation techniques – the use of fast-growing plants for remediation of soil and groundwater contaminated with organic and inorganic pollutants. The research team has worked on sites contaminated with persistent organics including TCE, carbon tetrachloride, methyl tertiary butyl ether (MTBE), formaldehyde, trichloroethane, ethylene dibromide, chlorobenzenes, and pesticides.

For example:

- A three-year field trial to determine the efficacy of phytoremediation using hybrid poplar trees to remove TCE from groundwater demonstrated that by simply planting the trees and letting them grow, that over 95% of the TCE was removed from test plots and that the trees completely degraded TCE to nontoxic substances and did not release TCE into the air.
- 13 years after 700 gallons of trichloroethane (TCA) were spilled in Medford Oregon, the researchers established a phytoremediation system – the first in the country to use a system where the contaminated water was pumped up and used as a drip irrigation water on the trees. While this technology will not clean up the site faster than conventional technologies, it does represent a significant cost savings.

- Despite conventional cleanup efforts following an accidental spill of the insecticide ethylene dibromide (EDB) in Hawaii, the contaminant percolated down into an underground reservoir used for drinking water. The research team tested a series of native Hawaiian plants and determined that Koa (*Leuceana leucocephala*) can tolerate the high levels of EDB at the site, can detoxify the contaminant, and can rapidly treat large amounts of groundwater. The groundwater remediation process continues.
- Strong public support for phytoremediation induced the Navy to consider the technology to remediate a TCE plume that was seeping out from a landfill and entering a nearby wetland. Even though there was extensive engineering required on the site to enable the trees to be planted, there was still a significant cost savings – with the phytoremediation project costing approximately $3.3 million vs. $10.5 million for conventional technologies.

The researchers are also developing additional transgenic plants, capable of significantly higher rates of metabolism of specific contaminants. By introducing a mammalian metabolizing gene (cytochrome P450) into tobacco plants, they have engineered plants with profoundly increased rates of TCE metabolism. The gene raises enzyme levels to such an extent that the plants removed approximately 600 times the amount of metabolites as the non-transformed plants. Research is also underway to identify and isolate genes from native plants involved in this degradation.

Phytoremediation is a cost-effective means to reduce the amounts and toxicity of toxic pollutants in soil and ground water that can reduce human exposures and improve public health. Furthermore, the use of plants to remediate contaminated soil and groundwater is an aesthetically pleasing, solar energy driven, passive technique suitable for a variety of contaminants and conditions. Currently, the United States spends more than $5 billion per year to clean up organic pollutants. *Gordon estimates that up to half of organic pollutants could be cleaned up using phytoremediation at a cost of only 25 - 30% of standard methods.*

[**Gordon (University of Washington)**]

4f.iii Microbial Degradation of PCBs

Scientists estimate that hundreds of millions of pounds of polychlorinated biphenyls (PCBs) have been released into the environment as the result of improper disposal practices and accidental releases. This is a significant environmental problem because PCBs are chemically and thermally stable, and complex PCB mixtures generally are resistant to biodegradation. As illustrated by the estimated $460 million cost of the five-year project required for the General Electric Company to dredge PCB-tainted sediments from the Hudson river bed, conventional PCB remediation methods are both expensive and time-consuming. Supported by the SBRP, James Tiedje at Michigan State University is investigating microbial processes that could be applied to the bioremediation of soils and sediments contaminated with PCBs.

A PCB is a molecule consisting of a biphenyl nucleus with chlorine at any or all of the 10 available sites – 209 different PCB congeners are theoretically possible. Although numerous biphenyl-degrading microbes are known, high numbers of chlorine substituents typically prevent aerobic microbial enzymes from attacking the aromatic rings of PCBs. Yet anaerobic microbial communities were discovered that possess reductive dechlorination activity, which replaces some of the chlorines on the biphenyl rings with hydrogen, leaving the biphenyl rings intact. The resulting partially dechlorinated PCBs are more susceptible to aerobic co-metabolism via ring opening, and chemical oxidation. Moreover, genetically enhanced microbes can be constructed to reclaim these PCBs into the global carbon cycle via their mineralization. Thus, the enhanced (bioaugmented) sequential anaerobic-aerobic treatment could result in the complete destruction of even complex PCB mixtures (Aroclors) and is potentially the most promising approach for bioremediation of PCB contaminated soils and sediments.

Tiedje and his colleagues are examining aspects of both the anaerobic and aerobic phases of this process, working to find information that can be used to overcome factors limiting the implementation of sequential anaerobic-aerobic biotreatment technologies. For example, naturally occurring anaerobic dechlorination of PCBs is generally incomplete. Building on previous work, the MSU researchers are studying the effectiveness of iron sulfate ($FeSO_4$) amendments to stimulate dechlorination of different PCB mixtures which may be found at Superfund sites. *Amendment with $FeSO_4$ is inexpensive and innocuous, and this technique has great potential to yield the maximum extent of dechlorination, which is crucial for the following aerobic PCB reclamation.*

Two distinct issues must be addressed with respect to maximizing the potential of the aerobic phase of PCB biodegradation. First, it is important to identify the persistent intermediates that accumulate during aerobic degradation and to estimate their potential toxicity. Second, research is needed to develop microbial systems that are capable of thriving in contaminated sediments and able to mineralize complex PCB mixtures thus preventing accumulation and release into environment of the toxic intermediates.

The results of this MSU research suggest that a sequential anaerobic-aerobic treatment scheme could be a beneficial remediation technology. However, further research is needed to resolve remaining questions. For example, how are the degradative processes affected and being affected by the metabolic networks in microbial communities? Recent developments in genomic sequencing and DNA microarray technology create opportunities for answering these questions at the metabolic, regulatory, community and populational levels. The MSU team is employing microarray techniques to understand complex processes involved in successful biodegradation within a microbial population, naturally occurring and/or engineered, by introduction of foreign members. Success

in this direction will help determine the practicality of the emerging recombinant technologies for remediation of environmental hazards.

This research advances our knowledge of the molecular, population, and environmental factors that impact the fate of PCBs at Superfund sites. *Sequential PCB transformation at anaerobic-aerobic interfaces, especially in sediments and soils, represents the greatest promise for remediation of large areas of low-level PCB contamination.*

[**Tiedje** (**Michigan State University**)]

4f.iv Microbial Transformation of Trichloroethene (TCE) in Anaerobic Groundwater

Jennifer Field at the Oregon Health and Science University is leading studies focused on enhancing microbial transformation of trichloroethene (TCE), a common toxic contaminant at Superfund sites. Natural degradation of TCE in groundwater can be quite inefficient and slow, and toxic metabolic products including vinyl chloride can accumulate if degradation does not proceed to completion.

Dr. Field and her colleagues are developing a technology that can stimulate *in situ* degradation rates and help prevent accumulation of toxic metabolites. Dr. Field's work involves the use of single-well "push-pull" tests in which chemical additives can be introduced to real-world contaminated groundwater sites, followed by removal of samples from the same well to allow monitoring for destruction of the contaminant and formation of metabolites. By developing a technology that uses trichlorofluoroethene (TCFE) as an effective and readily detected surrogate for TCE, Dr. Field has greatly improved the ability to predict the efficiency of TCE degradation in real-world contaminated groundwater sites. Her group has further demonstrated that adding simple, inexpensive organic substrates (such as lactate, fumarate or succinate) can stimulate the reductive dechlorination process in aquifers that destroys TCE, even where little or no reductive dechlorination activity has been previously observed.

Field work for this project is on-going at two sites with groundwater contaminated by TCE and its reductive dechlorination products:

- Richmond, CA, the site of a former chemical manufacturing facility, where Field's team is testing the hypothesis that chlorofluoroethene (CFE) can be added to groundwater by means of the single-well push-pull test followed by monitoring for the formation of fluoroethene (FE) as a probe for the analogous transformation of vinyl chloride (VC) to ethene. The focus of this field project is on VC transformation because it is the TCE transformation product of greatest concern and its transformation to ethane is considered a critical step in the remediation of TCE-contaminated groundwater.

- Homelite site in North Carolina – where Field is testing the hypothesis that TCFE can be used to detect biostimulation of indigenous reductively-dechlorinating microbial populations after the addition of lactate or fumarate to a TCE-contaminated aquifer.

By increasing the rate and extent of TCE biotransformation, the amount and toxicity of this groundwater contaminant can be effectively reduced, which will ultimately reduce the potential for adverse human exposures to occur. With further development and refinement, *Dr. Field's technology should one day result in the ability to test a TCE-contaminated site using TCFE as a surrogate, and then develop a recipe for an inexpensive, non-toxic chemical additive mixture that will dramatically speed up the rate of decontamination in comparison to currently existing remediation strategies.* These technologies are needed to improve the efficiency of groundwater remediation, which ultimately will result in reduced potential for human exposure.

[**Field (Oregon Health and Science University)**]

4f.v Sulfurhexafluoride (SF_6), a New Groundwater Transport Tracer

At many Superfund sites in the United States, it is critically important to be able to map the underground flows of groundwater. For example, it is essential to determine whether contaminated water is flowing toward a source of drinking water or a lake that is used for recreational purposes. Often, the flow of groundwater is complex – and not unidirectional. Thus, the ability to determine multidirectional flows and flow rates is fundamental to assessing the risks at any site.

To date, few studies have used sulfurhexafluoride (SF_6) as a purposefully injected inert tracer in groundwater studies. SF_6 has the advantage of being relatively easy to detect by gas chromatography with a dynamic range of at least five orders of magnitude, and it does not occur naturally in groundwater to any significant extent. The extremely low detection limit and the chemical stability of SF_6 make it possible to follow this tracer over very long periods of time without it impacting groundwater flow dynamics or geochemistry. Due to its high solubility in organic phases, SF_6 may be used as a partitioning tracer. Dr. James Simpson at Columbia University conducted two tracer experiments at a former municipal landfill site in Maine, characterized by elevated arsenic (As) concentrations. The purpose of the experiments was to evaluate the capture efficiency of injected fluids by an extraction well and to trace the zone of influence of *in situ* redox interventions.

During the first experiment, two potential tracers, i.e., SF_6 and sodium chloride (NaCl), were injected beneath the landfill via a single well. After 50 days, 90% of the tracer mass was recovered by a nearby extraction well, located about 15 meters down gradient, indicating high capture efficiency. The center of the tracer plume moved at a velocity on the order of one meter/day. NaCl concentrations fell quickly below the detection limit, while SF_6 could reliably be measured in the

extraction well and several monitoring wells. During the second experiment, SF_6, sodium bromide (NaBr), and a commercial oxidizing agent (primarily consisting of MgO_2) were mixed and then injected uniformly at a series of 10 points along a narrow zone (1 m by 10 m) over the entire depth of the aquifer (10 m) and monitored in a series of multilevel wells installed downstream of the injection zone. SF_6 and bromide passed by the monitoring wells at various depth levels in the multi-level wells in similar fashion. After seven months, about half of the mass for each tracer had been recovered, a substantially smaller fraction per volume of extracted groundwater than was recovered in the first injection experiment, probably as a consequence of continued retardation of tracers in low-permeability zones. *SF_6 breakthrough curves clearly defined the zone of influence of the redox injection and played a key role in the interpretation of the redox experiment. Thus, SF_6 holds great promise as a simple inert compound that can be used to map underground water flows.*

[**Simpson (Columbia University)**]

4f.vi The Use of Partitioning Tracer Tests for Characterizing Immiscible-Liquid Contamination at Hazardous Waste Sites

Soil and groundwater pollution has become one of our most pervasive environmental problems, and remediating contaminated sites has proven to be a formidable challenge. Risk assessments, as well as remediation efforts, are often limited by the complexity of the subsurface environment and by our limited knowledge of that environment. As noted in a National Research Council Report, advances in characterizing subsurface properties and processes are essential for improving subsurface assessments and cleanup technologies. The presence of immiscible liquids in the subsurface is often the single most important factor limiting remediation of sites contaminated by organic compounds. Unfortunately, because the subsurface distribution of immiscible-liquid saturation is complex, the use of traditional sampling methods is often problematic. The use of partitioning tracer tests for characterizing the occurrence, quantity, and distribution of immiscible-liquid saturation in subsurface systems has thus become of great interest.

Mark Brusseau at the University of Arizona is conducting a partitioning tracer test by injecting a solution containing two or more tracer compounds into the subsurface domain of interest. Immiscible-liquid contamination is measured by comparing the transport of a tracer that can partition into the immiscible liquid (partitioning tracer) to that of a tracer that does not (non-partitioning tracer). Generally, the organic-liquid contamination temporarily retains the partitioning tracer, which slows its transport compared to that of the non-partitioning tracer. Thus, retardation of the partitioning tracer indicates the possible presence of immiscible-liquid contamination within the zone through which the tracer solution moved (swept zone). When other potential sources of tracer retention, such as sorption by the porous media, are negligible or have been accounted for, the

magnitude of the retardation of the partitioning tracer is representative of the amount of immiscible liquid contamination present in the swept zone.

The magnitude of the observed retardation, and thus the calculated quantity of immiscible-liquid contamination, is a function of the areal extent of the tracer test (swept zone) and the volume of immiscible liquid in the swept zone. The scale of measurement corresponds to the size of the swept zone, which is controlled by the placement of the injection and sampling points. Thus, the tracer method provides a measure of immiscible-liquid saturation at scales larger than the traditional, point-sampling methods. By sampling a much larger volume of the subsurface compared to that measured with cores or monitoring wells, the partitioning tracer method has potentially a greater chance of encountering immiscible-liquid contamination and thus detecting its presence. This enhanced detection potential is a major advantage of the method. Other advantages of the method include no significant limits to the depth of application, and the ability to tailor the scale of measurement to the objectives of the study. *The results of these tests indicate the partitioning tracer method can provide information extremely useful for site characterization and risk assessment, and for evaluation of remediation system performance.*

[**Brusseau (University of Arizona)**]

4f.vii Arsenic Mobilization in Bangladesh Groundwater

In 1993, an epidemic of arsenic poisoning began to emerge in Bangladesh. The epidemic is the indirect consequence of a well-intentioned campaign by donor agencies approximately 20 years ago to shift water consumption patterns in Bangladesh away from microbial disease-ridden surface water to "safe" clean tube well ground water. That epidemic is now known to expand into other areas of Asia, including India and Vietnam. Health experts estimate that 40 million people have been chronically exposed to arsenic, making this the worst environmental health disaster of the 20th century.

Yan Zheng leads a multi-disciplinary group of scientists at Columbia University working in Bangladesh to try to understand the reasons why arsenic is so rich in Bangladesh groundwater. As in many other regions where arsenic is a problem in drinking water, the earth itself is not especially rich in arsenic. There is something about the water that allows arsenic to elute from the minerals in the soils and become soluble in drinking water. Delta regions appear to be particularly susceptible. *This group has determined that groundwater in Bangladesh is very unusual in that it contains virtually no free oxygen.* They believe that the bacterial decomposition of deltaic sediments, i.e., the organic material such as leaves and plant materials that deposit in the basins of the great rivers of the region, consumes all of the oxygen in the water. *The resulting "reducing" water causes arsenic to be chemically reduced*

from arsenate (As^{+5}) to arsenite (As^{+3}), thereby making it soluble in water and thereby posing a hazard to human health. They have also determined that drinking water concentration does not change with time or season, i.e., the flood and dry seasons in Bangladesh.

Zheng's group has also been working with the United Technologies Co. and the USEPA at a Superfund site in Winthrop, Maine (a former municipal garbage dump) where arsenic levels are high in the groundwater. Columbia scientists have determined that the chemistry of arsenic in groundwater in Bangladesh is very similar to that in Maine. At the Winthrop site, rather than bacterial decomposition of river sediments, it is the bacterial decomposition of garbage that leads to the consumption of all of the oxygen in the water and the appearance of arsenic in groundwater.

The Columbia group is working with the above parties to try to inject an oxygen-containing compound into the groundwater to determine whether this may eliminate the appearance of arsenic in the water.

[**Zheng (Columbia University)**]

4f.viii Monitoring *In Situ* Bioremediation of TCE

In situ bioremediation of chlorinated solvents can occur by microbial reductive dehalogenation. For trichloroethylene (TCE), this involves the sequential reduction of TCE to dichloroethene (DCE), followed by conversion of DCE to vinyl chloride (VC), and finally VC to ethene. Under anaerobic conditions, the microorganisms that catalyze these reactions use the solvents as electron acceptors while using a range of substrates (e.g., lactate, methanol, H_2) as electron donors/energy sources. The most serious challenge associated with the use of reductive dechlorination as a TCE remediation option is that the process does not always continue to ethene, resulting in the accumulation of DCE and VC. Both are hazardous compounds, and VC is a known human carcinogen.

Supported by the SBRP, Lisa Alvarez-Cohen of the University of California, Berkeley and Mark Conrad of E.O. Lawrence Berkeley National Laboratory are directing a research program with the goal of developing tools to evaluate the progress of *in situ* bioremediation of TCE. The Berkeley team is taking a two-pronged approach, as they work to develop:

- Stable carbon isotope analysis methods to monitor the microbial conversion of TCE to ethene
- Non-culture-based tools to monitor microbial population dynamics and community structure during the degradation of chlorinated solvents

Stable carbon isotope analysis: A major barrier to implementation of *in situ* bioremediation techniques has been the lack of reliable field monitoring tools. Bioremediation processes are complex and occur in subsurface, heterogeneous environments. Consequently, it is difficult to distinguish

between contaminant concentration changes resulting from biodegradation and those that occur due to physical processes such as groundwater transport or mixing. As a result, complete stoichiometric conversion of TCE to ethene can rarely be demonstrated in the field.

Drs. Alvarez-Cohen and Conrad are applying the technique of stable isotope fractionation to monitor the transformation of TCE and its degradation products. Degradation of organic compounds by biological enzymatic processes can cause significant shifts in the ratio of carbon isotopes ^{13}C to ^{12}C (delta-^{13}C values) in both the reactants and products. Because of its lighter mass, ^{12}C is more weakly bonded and reacts more readily than ^{13}C. During dechlorination of TCE, ^{12}C bonds are preferentially degraded, resulting in isotopic enrichment of the residual contaminant in ^{13}C. Analysis of relative abundances of ^{12}C and ^{13}C yields information needed to assess rates of TCE degradation.

During a pilot study at the Idaho National Engineering and Environmental Laboratory's field site Test Area North, designed to investigate the treatment potential of using lactate to stimulate *in situ* biologic reductive dechlorination of TCE, the researchers conducted detailed, time-series stable carbon isotope monitoring. *The results of this pilot study clearly demonstrate the sensitivity of carbon isotope measurements to the processes that occurred during the enhancement experiment.*

Quite significantly, this study also established the value of lactate enrichment to act as a substrate for microbial reductive chlorination. The remediation technique yielded an estimated $15 million cost savings to the DOE for cleanup of the site.

Non-culture-based tools: Because over 99% of subsurface microorganisms are non-culturable, traditional culture-based techniques for evaluating subsurface communities are inadequate. To characterize community structure and to evaluate the relative contributions of different physiological groups of bacteria to the degradation of chlorinated solvents, Dr. Alvarez-Cohen is applying a series of non-culture-based molecular methods including:

- Clone library construction/clone sequencing
- Terminal restriction fragment length polymorphism (T-RFLP) analysis
- Fluorescent *in situ* hybridization (FISH) with rRNA probes
- Quantitative polymerase chain reaction (Q-PCR)

The research team initially used these techniques to study lactate-enriched microbial cultures from contaminated soil obtained from Alameda Naval Air Station. This was the first reported study where both PCR-based and FISH-based 16SrRNA methods had been applied in combination to characterize mixed communities performing reductive dechlorination. *This study demonstrated that used in combination, the suite of molecular techniques is a powerful tool for not only characterizing*

complex communities but also for tracking and monitoring specific microbial species in environmental systems.

Application of the non-culture based tools provided information on the ecology, physiology, and biochemistry of microbial communities involved in complete reductive chlorination, and on perturbed communities capable of only partial dechlorination – *this knowledge can be applied to develop effective anaerobic treatment strategies for remediation of Superfund sites contaminated with chlorinated ethenes.* Expanding our capabilities to monitor and predict *in situ* bioremediation rates through the use of innovative isotopic and molecular tools will greatly increase our ability to apply this cost-effective and efficient technology in the field, leading to extensive cost savings and risk reduction.

[**Alvarez-Cohen (University of California – Berkeley)**]

4f.ix Structure and Function of Microbial Communities in Bioremediation

Many Superfund sites contain soils and sediments contaminated with toxic heavy metals and aromatic hydrocarbons. In most cases, the volume of these materials and associated treatment costs precludes highly engineered cleanup solutions. The main organic-degrading and metal-transforming processes in contaminated environments are carried out by indigenous microorganisms. SBRP investigators at the University of Cincinnati, led by Paul Bishop and Jodi Shann, are using a multidisciplinary approach to investigate the structure and function of these microbial communities, and to develop methods for maximizing their usefulness in bioremediation.

The collaborative research by SBRP investigators at the University of Cincinnati has led to a better understanding of the types of microorganisms that are important in biodegradation, the role that plants play in the activity of these microorganisms, and the structure of biofilms of degradative microorganisms. Ultimately, these studies will be expanded into the development of improved bioremediation methods.

One aspect of this research has focused on characterizing the bacteria actually involved in biodegradation, using a variety of traditional and molecular methods. The ultimate value of *this increased understanding of the ecology and genetics of these bacteria is that this information can be used to increase the efficiency and efficacy of bioremediation efforts, and ultimately to reduce potential human exposure to these genotoxic compounds.*

Dr. Shann is testing the hypothesis that plants increase the remediation of soil by the uptake of metals (phytoextraction) and/or by the release of exudates that influence soil microorganisms (rhizosphere degradation). Although the studies on uptake and bioavailability are important, it is the

work on root exudates that will have the greatest impact on the development of phytoremediation as a technology. This project has provided clear evidence that root exudates are responsible for a majority of the "rhizosphere effect" – the changes in microbial community structure and activity that are responsible for the enhanced degradation of PAHs observed in planted soil.

Dr. Bishop has made important advances in characterizing biodegradative microbial communities through the elucidation of the structure and function of biofilms. Microorganisms tend to aggregate into biofilms on solid surfaces. The primary intent is to develop a fundamental understanding of the role of biofilms in soil bioremediation systems, in terms of biofilm growth and structure, and the role of the biofilm on mass transport to the biofilm microorganisms responsible for the contaminant biodegradation. A particularly salient aspect of this research is that it is addressing not only bioremediation of individual compounds, but also mixtures of toxic compounds with a range of recalcitrance. *Microelectrodes have been developed and used to monitor biological activity within the biofilm, and results of these studies have provided new and important information about the structure and dynamics of these microbial communities. Information gained in these studies is now being used to design bioreactors that can be used to enhance biodegradation at hazardous waste sites.* In addition, the microelectrode technology is being adapted for use in the field.

[**Shann/Bishop (University of Cincinnati)**]

4f.x The Role of Bacteria in Bioremediation of Metals

Groundwater is a critical resource – providing water for drinking, industry and agriculture, as well as sustaining surface waters, wetlands, and the ecosystems they support. Accumulation of toxic metals in water supplies is an extremely serious environmental problem, and a costly one to remediate.

As part of their natural activity, some bacteria can detoxify or immobilize heavy metals. To enable environmental engineers to design more suitable cleanup technologies for contaminated sites, Brad Tebo leads a team of researchers at the University of California, San Diego (UCSD) in studies to examine the mechanisms used by bacteria to detoxify environmental contaminants. The research is focused on increasing our understanding of the processes by which bacteria are able to carry out these reduction/oxidation reactions, and to identify specific genes and proteins responsible for the biochemical activity.

They are evaluating two different biological strategies to reduce the toxicity of heavy metals:

- Direct biological transformation of toxic metals into a less toxic form. Specifically, the UCSD researchers are investigating the reduction of the mutagenic and carcinogenic form of chromium, hexavalent chromium (Cr(VI)), to the less toxic Cr(III).

- Scavenging and immobilization of toxic metals on the surface of highly reactive, enzymatically produced, metal oxides. Specifically, Tebo's group is investigating the mechanisms involved in bacterial transformation of soluble manganese (Mn(II)) to the insoluble oxides Mn(III) and Mn(IV). Mn oxides are strong oxidants, and have negatively charged surfaces and high surface areas. As a result, heavy metals such as arsenic, cobalt, copper, lead, nickel, and zinc can either be adsorbed onto the surface of the oxides or incorporated into their crystal lattice – effectively locking them in the solid matrix of soils and sediments and removing the metals from the water.

The UCSD Cr(VI) reduction studies are concentrated on isolating and characterizing Cr(VI) reducing bacteria from contaminated waters, soils and sediments, and examining the biochemical basis for Cr(VI) reduction. Not only will identification of these mechanisms provide valuable information for the development of bioremediation processes, but Dr. Tebo believes that identification of the genes and proteins expressed in response to Cr(VI) could lead to the development of biomarkers of environmental Cr(VI) exposure.

Tebo's laboratory determined that when Cr(III) is bound into solution as it is being produced, the ability of the bacterium to withstand toxic levels of chromium is greatly increased. They are continuing to study this trend, and are trying to determine if the compound that binds to Cr(III) is of biological origin.

Manganese cycling between its solid and soluble forms is largely driven by microbial activities, and the UCSD researchers are working to discover the mechanism of Mn(II) oxidation. They have demonstrated that the protein involved in Mn(II) oxidation is one of a family of multi-copper oxidase proteins. In addition, they have:

- Isolated a number of spore-forming bacteria in coastal marine sediments that produce dormant spores which enzymatically oxidize soluble Mn(II) to insoluble Mn(IV) oxides. *These are the first active Mn(II)-oxidizing enzymes identified in spores or gram-positive bacteria, and this is also the first enzyme of its kind identified in a marine bacterium.* This work suggests that the commonly held view that bacterial spores are merely inactive structures in the environment should be revised.
- Determined that the Mn(II)-oxidizing enzyme (Mn(II) oxidase) is also capable of oxidizing certain phenolic compounds. This suggests that the enzyme could play a role in strategies for dual remediation of organics and metals. The researchers are currently devising a means to produce sufficient quantities of the Mn(II) oxidase to study the biochemical details of Mn(II) oxidation.

Heavy metal contamination is widespread in the United States – threatening water supply sources as well as pristine watersheds. Harnessing naturally occurring processes for the purposes of remediation is a far more cost-effective proposition than physically removing non-degradable contaminants such as heavy metals. Stabilizing and transforming toxic metals to environmentally benign forms is currently being explored at a variety of Superfund sites with some success. A detailed scientific

study of the mechanisms involved at the molecular level will help refine the current strategies and render them more effective.

[**Tebo (University of California – San Diego)**]

4f.xi Transport, Enzymatic Regulation and Microbial Transformation Rates in Flowing Groundwater Systems

This project brings together two well-established investigators having complementary expertise. Dr. Abriola, an environmental engineer, has developed mathematical models predicting the flow of volatile contaminants in and through the subsurface. She is collaborating with microbiologist and bioremediation expert Dr. Kukor in this project designed to understand factors in the soil and groundwater (the porous subsurface) that impede or increase the rate of bacterial degradation of common volatile contaminants. Volatile solvents, often found in soil, are known to reach aquifers used for drinking water. This combination of expertise is yielding information that permits the development of tools that will permit a good estimate of the time (months to years) it takes for bacteria naturally present in the subsurface to degrade volatile organic solvents that are in the subsurface from spills or leaking from underground storage containers at Superfund Sites. The predictive tools to be developed are mathematical models that simulate the movement and disappearance of contaminants in the subsurface. The accuracy of the predication that can be made using these models depends on the correctness of our understanding regarding the factors that control the ability of bacteria to degrade pollutants to non-toxic products. This project is devoted toward increasing our understanding of the fate of solvents in the subsurface, and using that information to build better and more accurate predictions of the rate at which bacterial-based degradation or "cleanup" occurs in the subsurface. Accurate knowledge of the time of residence of toxic contaminants in the subsurface is a major factor in decision-making regarding a contaminated site. For example, a site may be sequestered or fenced off to reduce toxic exposures to humans and wildlife when the rate of disappearance of the contaminant is sufficiently rapid. On the other hand if it is very slow, procedures must be taken to increase the rate of degradation by the use of a variety of modifications and treatments that can be applied to the site.

Major portions of the information needed to understand those factors important in controlling the bacterial degradation of solvents in the subsurface come from experiments performed in the laboratory under controlled conditions. Early work in this project by Dr. Kukor investigated the genetic factors controlling the amount of enzymes in a bacteria known to be involved in degrading several different volatile solvents such as toluene, benzene and trichloroethylene (or TCE). The biochemical nature of the degradation pathway was studied, and the genes involved in the processes were identified. The conditions needed for stimulation of enzyme-based degradation and those

conditions that impede it were elucidated. For example, it was found that high concentrations of TCE in the soil can stimulate bacteria to produce more degrading enzymes. However, at lower concentrations of the solvent this stimulation is not present, making the disappearance of low concentrations very slow. Also, it was found that particular proteins produced by the bacteria were found to be stimulators of the process for production of the enzymes needed for contaminant degradation. The investigators are now examining methods to increase that protein in the presence of low concentrations of TCE.

In other studies, the presence of toluene, a common solvent often found in soil contaminated with TCE, was found to stimulate an enzyme that degrades TCE as well as the toluene itself. However, some of the products produced as a result of toluene degradation decrease the degradation of TCE making the control of TCE degradation multifaceted and complex. More recent results have indicated that the contaminating solvent or perhaps its degradation products can alter the production of substances in bacteria that augment their attachment to soil particles, and this can be another factor controlling the rate of TCE degradation. This type of information and many other segments of information from research will eventually permit a sufficient understanding of the factors controlling contaminant degradation in the subsurface. At that point, it will be possible to develop accurate estimates, using advanced models of the time course, for removal of contaminants given a set of circumstances that exist at a contaminated site.

It should be noted that the basic science connected with this project should not be considered as only pertaining to the degradation of solvents and the remediation of contaminated soil and groundwater. The new information gained regarding the fate and action of a specific bacteria present in the subsurface may apply to other types of bacteria. As such, the information may be used in situations in which we need to control a harmful action of soil bacteria. In that case, knowledge of the factors controlling that harmful action may permit development of strategies to eliminate the action produced by the organism or by controlling the growth of the bacteria itself. Alternatively, the production by bacteria of specific substances, e.g., antibiotics, that are beneficial to health can be increased using knowledge of the control of specific bacterial genes.

[**Abriola (University of Michigan), and Kukor (Rutgers University)**]

4f.xii Mechanisms of Bioavailability Regulation in Soil

The focus of Fred Pfaender's SBRP-funded research at the University of North Carolina–Chapel Hill has been to develop an understanding of the possible physical, chemical, and biological processes that regulate the bioavailability of polycyclic aromatic hydrocarbons (PAH) in soil. Clearly, all of these processes are important but vary from site to site.

Pfaender and his colleagues recently found that *when contaminated soils are incubated under anaerobic conditions, the PAH contaminants become much more extractable and indeed much more water-soluble.* They have termed this "anaerobic mobilization". Anaerobic mobilization occurs when anaerobic microorganisms utilize the hydrogen ions in the soil pore water. This results in an increase in soil pH. At higher pH, the organic matter of the soil becomes more soluble. As the organic matter goes into solution it appears to take sorbed PAH along with it, making them more soluble. In their solubilized form, PAHs appear to be biodegradable. The addition of electron acceptors for the anaerobic microbes stimulates the process. This phenomenon has not been observed previously.

The development of this process offers the potential for new approaches to remediation of sites contaminated with aged (long-term) PAHs. The successful implementation of this process could reduce environmental concentrations of PAH and therefore could reduce exposure. This is an example of how fundamental research into soil microbiology and chemistry has yielded ideas that have the potential to become valuable remediation processes.

Pfaender and his associates at UNC-CH recently received an STTR grant from the SBRP to develop bioremediation strategies based on their observed anaerobic mobilization of the PAH in soil. One of the major problems with bioremediation of highly non-polar contaminants is that they rapidly become unavailable to the microorganisms whose biodegradative capacity is needed to break down the contaminants. This is the core of the Phase I STTR project jointly conducted with Chatham Research Limited of Research Triangle Park, North Carolina. During the first two years, the objectives focus on documenting the phenomenon in soils from contaminated sites around the country, establishing the optimal conditions for generating and maintaining anaerobic conditions in soil, optimizing the types and amounts of electron acceptors to add, and developing processes for the *in situ* or *ex situ* degradation of the mobilized contaminants. Phase II will be done in collaboration with RETEC Inc., an environmental consulting firm, to conduct field-scale tests of the process.

[**Pfaender (University of North Carolina – Chapel Hill)**]

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4g. Contributions to Human and Ecological Risk Assessment Processes

The goal of every hazardous waste site cleanup is the protection of the public's health and the environment. However, because it is impossible to quantify all risks, site managers use data provided from the risk assessment process to define exposures of concern and potential threats. The more robust the risk assessment, the better able the site managers are to make cost effective, yet protective, choices.

The SBRP is conducting research with the goal of enhancing the data available for risk assessment models. Studies conducted by the Program provide valuable information about biological mechanisms and toxicity. Other studies are developing novel biomarkers that can be used for assessing exposure. Knowledge and understanding at this level have the potential to improve the accuracy of risk assessments driving site remediation decisions.

Another unique aspect of the SBRP is its work on ecological research. The SBRP sponsors scientific inquiry to determine how a better understanding of ecological impacts from hazardous waste sites can further our understanding of potential human health effects. This creative, holistic approach should help the USEPA to better integrate the presently separate ecological and human health risk assessments conducted at hazardous waste sites into more comprehensive site models.

These data have the potential to contribute to the risk assessment process. The SBRP is committed to ensuring that its data are readily available to environmental engineers, risk assessors and site managers. Over the years, we have learned from professionals conducting site assessments that peer reviewed literature is not the most effective mechanism of communicating our science to them. With this community in mind, as discussed in Section 2, we have been proactive in implementing creative and effective communication tools, including the *Research Briefs*, *RISKeLearning*, and our web page.

Below we present examples of SBRP projects that have led to reduced uncertainty in the risk assessment process by providing new tools, new approaches or filling data gaps.

4g.i Development of a Biomarker of Exposure to Benzene

Benzene is a ubiquitous environmental contaminant – it has been detected at over 800 Superfund sites. According to the USEPA's Toxics Release Inventory, from 1987 to 1992, releases of benzene to water and land totaled over 2 million pounds – these releases were primarily from petroleum refining industries. Environmental contamination also occurs as the result of benzene emissions to air from chemical and manufacturing processes, from discharges into water from industrial effluents, and from combustion of most fuels. Individuals may be exposed to benzene by consuming contaminated

water or by inhaling motor vehicle exhaust or evaporation at gasoline service stations. Tobacco smoke accounts for approximately 50% of the public's exposure to benzene.

Benzene is both toxic and carcinogenic – there is strong evidence that it causes various types of leukemia and other blood diseases. Though scientists have studied benzene for over 100 years, much remains to be learned about the metabolism and mechanisms of carcinogenicity of this deceptively simple molecule.

Following exposure, ~20% of benzene is exhaled in the breath (BB). The remaining ~80% is absorbed, metabolized, and may be excreted as a variety of urinary products, including phenolic compounds (i.e., phenol, hydroquinone, catechol and trihydroxybenzene), t,t-muconic acid (MA) and S-phenylmercapturic acid (SPMA), as well as unmetabolized urinary benzene (UB). Although the mechanism of benzene carcinogenicity is not known, it is thought to involve the actions of one or more metabolites, notably hydroquinone that is converted in the body to 1,4-benzoquinone.

Steve Rappaport and a team of scientists at the University of North Carolina, Chapel Hill (UNC) are conducting studies to develop and validate biomarkers of exposure to benzene with which to investigate exposure-response relationships in humans. The UNC researchers have developed simple and sensitive assays for UB and BB among occupationally exposed workers and the general public. These biomarkers include protein adducts of several toxic metabolites of benzene, of benzene in breath and urine, and of benzene metabolites in urine. Using these biomarkers, they are evaluating the metabolism of benzene over a wide range of exposures in humans and rats. Human biomarkers are measured in samples of blood, urine, and breath obtained by collaborators at other institutions and in prior studies. Metabolites of interest include benzene oxide (BO) and 1,4-benzoquinone (1,4-BQ).

The researchers have analyzed levels of benzene oxide-albumin adducts (BO-Alb) from occupationally exposed workers and unexposed controls from Shanghai, China. The scientists observed a strong effect of benzene exposure and infer from the data that production of benzene oxide was less than proportional to benzene exposure (supralinear). Since benzene is a substrate for the cytochrome P-450 enzyme CYP2E1, these results are consistent with saturation of CYP2E1 metabolism, commencing in the range of 10 ppm.

In a more extensive investigation of BO-Alb and the albumin adduct of 1,4-benzoquinone (1,4-BQ-Alb), supralinear adduct production was also observed with increasing benzene exposure among exposed workers and unexposed controls in Tianjin, China. The transition from linear to saturable metabolism began at about one ppm. Adduct levels were generally lower in older workers, indicating that benzene metabolism diminished with age, at about 2% per year of life. The levels of both BO-Alb and 1,4-BQ-Alb were elevated in Chinese control workers who smoked compared to

nonsmokers. Using linear low-exposure-adduct relationships, Rappaport predicted that less than 1% of the observed adduct levels arose from ambient exposures to benzene.

Because the researchers observed supralinear production of adducts of benzene metabolites with benzene exposure greater than 1 ppm, *the common assumption of linear kinetics at much higher benzene exposures could lead to substantial underestimation of leukemia risks.*

The significant reduction in production of these adducts among older workers in the same study also presents the strongest evidence to date that benzene metabolism diminishes with age among healthy persons.

[**Rappaport (University of North Carolina – Chapel Hill)**]

4g.ii Sources, Transport and Fate of Arsenic in Groundwater

Scientific and practical advances resulting from SBRP-funded research have had a major impact both on our mechanistic understanding of the effects of arsenic on human health, as well as on public health policy leading directly to improvements in public health. *The Dartmouth geochemistry and epidemiology groups worked together to comprehensively document the extent of arsenic contamination in New Hampshire, which in turn led directly to better public health assessment by the State of New Hampshire and Region I USEPA.* In a survey of several thousands of private wells, the research team found that one in four has arsenic in excess of 5 parts per billion (ppb).

The geochemistry group, led by Joel Blum, also *found that the groundwater arsenic was derived principally from its natural occurrence in certain types of granite prevalent in New England bedrock, and that there was little or no contribution from surface anthropogenic sources,* contrary to previous hypotheses. This had a direct impact on risk assessments for groundwater contamination at several Superfund and other waste sites in New England which happened to be situated over natural sources of arsenic, and raised the issue of how to define "background" when considering cleanup of a particular site.

Margaret Karagas directed an epidemiology study that *yielded results which indicate that there may be as much as a 2- to 3-fold increased risk of skin and bladder cancer for New Hampshire residents drinking arsenic at or above 50 ppb.*

The 50 ppb level – the USEPA's drinking water standard since the 1940's – was lowered to 10 ppb in 2001 following several years of intense scientific and public policy debate. The early results of the Dartmouth epidemiology study, which is the only United States population-based case-control study linking individual arsenic exposures (measured in toenail samples) with cancer risk, was *one of the key studies that factored into that debate.*

One consequence of the Dartmouth SBRP program and its arsenic research receiving national attention during the arsenic debate was a significant elevation in the level of discussion about arsenic at the state and regional level. The Dartmouth program initiated a series of meetings with other state and regional groups interested in arsenic research and its application to public health. This in turn resulted in the formation of the New Hampshire Arsenic Consortium, which consists of the Dartmouth SBRP program, the NH departments of Health and Human Services and Environmental Services, the NH branch of the ATSDR, the NH branch of the U.S. Geological Survey, and the Region I USEPA office. These research and regulatory agencies meet regularly to discuss ongoing arsenic studies, plan future collaborative projects, and discuss coordinated application and dissemination of their results for the public health benefit.

[**Blum/Karagas (Dartmouth College)**]

4g.iii DNA Adducts as Biomarkers of Exposure and Effect

Risk assessments of National Priority List chemicals are usually based on linear extrapolation of tumor data from high exposures in experimental animals. The 1996 Revised Cancer Risk Assessment Guidelines contain many important changes regarding how future risk assessments will be performed, including the incorporation of more science on the mode of action and dose response of the chemical in question. SBRP-funded researchers at the University of North Carolina at Chapel Hill (UNC), led by Dr. Jim Swenberg, are working to develop a sound scientific understanding of the mode of action and the observed and expected dose response relationship of several of the major hazardous chemicals on National Priority List. Their data will be suitable for use with the revised guidelines and will improve the accuracy of the risk assessments driving site remediation.

The UNC researchers believe that several aliphatic and aromatic chlorinated compounds share a common mode of action - enhanced oxidative stress – which results in indirect damage to DNA and activation of signal transduction pathways involved in gene regulation and cell proliferation. Other members of this group of chemicals cause direct DNA damage. Currently, they are conducting studies to develop biomarkers of exposure and effect to both groups of chemicals. *These biomarkers will allow accurate determination of the amount, or molecular dose, that damages DNA and proteins; will enhance examination of the extent and type of both direct genotoxicity and oxidative stress related DNA damage; and will enable risk assessors to more accurately predict risk for cancer.*

Vinyl chloride (VC) has been detected at over 100 Superfund sites and is ranked fourth on the ATSDR list of hazardous substances. VC is present in soil, groundwater, aquifers, and wells as the result of inappropriate disposal, accidental spills, and as a product of incomplete biodegradation of chlorinated chloroethenes such as TCE. VC-induced carcinogenicity is believed to occur by

genotoxic mechanisms. The enzyme cytochrome P450 oxidizes VC to 2-chlorothylene oxide (CEO), which in turn alkylates to DNA to form 7-(2-oxoethyl)guanine (OEG) and a variety of promutagenic exocyclic base adducts. Although OEG is the major DNA adduct formed by VC, it does not cause mutations. N(2),3-ethenoguanine (N_2-,G) is formed in relatively small amounts, but has been determined to be both persistent and mutagenic. Scientists in Dr. Swenberg's laboratory have developed a highly specific and sensitive assay for N_2-,G (IA/GC/ECNCI/HRMS - immunoaffinity, gas chromatography, electron capture negative chemical ionization, high resolution mass spectrometry) and they are using N_2-,G as a biomarker to investigate several aspects of VC mutagenicity.

While it has been demonstrated that VC induces hepatic cancer in humans and rodents, neither experimental nor epidemiologic studies have established a definitive link between VC and brain cancer. By examining the molecular dosimetry of N_2-,G in rat brain and liver cells following exposure to VC, Dr. Swenberg's research team found clear evidence of genotoxic attack of hepatic DNA, but determined that N_2-,G is not formed in brain due to VC exposure. The same DNA adduct, N_2-,G, is present in both liver and brain DNA as a result of endogenous oxidative stress in both control and VC-exposed tissues. While VC does distribute to brain, it is not metabolized to CEO in brain. Likewise, CEO formed in the liver is not transported to the adult rat brain due to its extreme instability. By exposing rats to stable isotope-labeled VC, [13C2]-VC, the researchers were able to measure both the endogenous N_2-,G and the [13C2]-N2-,G in the same animals. These findings indicate that it is unlikely that N_2-,G or other VC-induced DNA adducts play a significant role in initiating carcinogenesis in the brain after exposure to VC.

These studies strongly support a causal role for N_2-,G in VC-induced carcinogenesis and suggest that the molecular dosimetry of N_2-,G in liver is predictive of cancer risk. The data do not support VC as a causative factor for the induction of brain tumors. This information also provides mechanistic support for the decision to include an additional 2-fold protection factor for young populations in the recent USEPA VC risk assessment.

[**Swenberg (University of North Carolina – Chapel Hill)**]

4g.iv Organochlorine Pesticides and Developmental Mortality

Several recent studies have documented evidence of reduced fertility, reduced fecundity and abnormal sexual development in wildlife in discrete locations around the world. These effects were found in alligators and fish in several central Florida lakes. It was proposed that this was linked to endocrine-disrupting effects of chlorinated Superfund chemicals. Tim Gross at the University of Florida's (UF) SBRP program is examining this hypothesis, developing sensitive biomarkers of

endocrine-disrupting effects, and seeking links between evidence of endocrine disruption, exposure to chemicals and reproductive effects in alligators and largemouth bass.

This study is important because it is well documented that adverse effects on sensitive species may serve as surrogates for health effects in humans. There is continued uncertainty about exactly how these findings apply to human health, but it is clear that compounds that produce such profound effects on wildlife will have ecological effects that will eventually impact humans. For example, disruption of reproductive function in food-producing species has implications for the human food chain. Also, direct effects of these chemicals may occur in humans, particularly in sensitive sub-populations. For example, it is known that infants and children are more susceptible than adults to many of the adverse effects of chemicals.

Since 1995, Gross has worked to advance our understanding of this complex problem. He has found higher levels of chlorinated pesticides, including DDE, toxaphene, dieldrin and chlordanes in alligators and fish from impacted lakes than from reference sites. His team of scientists has shown that alligator and fish females with high levels of pesticides have a variety of health effects related to reproduction and the development of the offspring. Gross documented reduced clutch sizes in alligators, and failure of embryos to develop normally. In addition, the researchers found that some fish failed to spawn, and those that did often produced juveniles that died during development.

Gross and his colleagues were able to link the presence of pesticides to the flooding of land previously used for farming. This finding has been communicated to risk assessors at the Florida Department of Environmental Protection, and changes have been made in the practice of flooding farmland such that levels of pesticides are determined and land remediated if necessary prior to flooding.

SBRP-funded research conducted at UF has also resulted in the development of several molecular tools for monitoring the effects of endocrine-disrupting environmental chemicals:

- Monoclonal antibodies have been developed to a protein (vitellogenin, which is important in reproduction) of several fish species, including striped bass, carp, killifish, gulf sturgeon, sheepshead minnow and brown bullhead. Two of these antibodies have been made into ELISA kits and are available to other researchers for monitoring environmental estrogens in naturally exposed fish.
- Other tools developed more recently include directed cDNA arrays targeted to endocrine disruptors with estrogenic effects. A micro-array of 200 genes selected because they were differentially expressed in fish exposed to steroid hormones (estradiol, progesterone, testosterone) has been developed and validated with laboratory-exposed fish. The array is being expanded to 300 genes. In the next few years, this product will be ready for use in environmentally exposed fish.

These new sensitive tools have the potential to impact public health and influence decision-making at Superfund sites by providing rapid methods of determining whether or not endocrine-disrupting chemicals

are present. The tools can be used for initial screening of sites, prior to more extensive chemical analysis. They can also be used to monitor cleanup of previously impacted sites.

[Gross (University of Florida)]

4g.v Application of Wildlife Biomarker Technologies in Remediation Decision-Making

Wildlife inhabiting chemically contaminated environments can serve as front line indicators of pollutant levels and potential health impacts. Wildlife biomonitoring at Superfund sites provides valuable data to support the design of remediation plans. These data can result in substantial savings of limited remediation resources while maximizing preservation of important natural areas and supporting effective remediation of the site. SBRP-funded researchers have developed biochemical, physiological, histopathological, and behavioral biomarkers in birds and mammals inhabiting contaminated sites to indicate chemical exposures and chemical effects.

As the science of biomarker technology evolves, researchers are finding additional and valuable applications. Mike Hooper and a team of researchers at Texas Tech University, a part of the University of Washington SBRP, are applying biomarker methods developed in SBRP-funded projects to develop health-based exposure and effects data for wildlife. At a Superfund site in Alabama, contaminated with a mixture of DDT/DDE and mercury, they conducted field studies to test the hypothesis that certain biomarker levels are a function of contaminant concentrations. If true, this would allow remediation professionals to predict levels of contaminant residues and effects in resident wildlife populations based on concentrations measured in soil and diet samples.

The scientists conducted their research in a hardwood bottomland floodplain (a seasonal swamp) that is on the National Priority List. The site owners had conducted extensive soil analyses and the SBRP researchers incorporated these data into a geographic information system (GIS) to develop a geographic distribution of DDT, DDT metabolites and mercury soil contamination. The scientists placed approximately 200 nest boxes throughout the study site. Birds, as indicator species in waste site assessments, can yield significant information on trophic (food chain) transport and toxicity of contaminants for use in remediation decision-making. Cavity nesting species occupy a variety of trophic positions and habitats, and allow direct monitoring of adults and developing young. The SBRP researchers studied prothonatory warblers (*Protonotaria citrea*), using radiotelemetry to triangulate specific foraging positions and ranges for both male and female adults. The scientists collected diet, egg, and nestling tissue samples and analyzed each for of DDT, DDT metabolites, and mercury. These data were then added to the GIS program for comparison with soil contaminant distributions.

The study site has heterogeneous contaminant levels as the result of seasonal flooding and topography modifying the original contaminant deposition areas. The unique design of this project allowed the researchers to control for variables that often confound this type of study. The scientists were able to match foraging areas to specific nesting pairs and use only soil concentration data from the appropriate foraging locations in their analyses of trophic-level transfer of contaminants. The researchers learned that while male and female prothonatory warblers fed at similar rates, the females tended to use larger foraging ranges. They therefore corrected their data to incorporate the feeding areas of both the adult male and female warblers.

The results of this study suggest that residue concentrations in bird tissues can be reasonably predicted from residue concentrations in the soil. The study demonstrated that warblers accumulated renal mercury from their diet at levels that correlated with soil and sediment levels. The researchers also found that soil concentrations of DDT and its metabolites were effective in describing the variation of contaminants in adipose tissues.

The project is an example of the technology transfer process that SBRP strives to promote. Basic experimental methods developed and characterized in the course of SBRP-funded research were successfully deployed in a real world application. *The findings of this research were incorporated into ecological risk assessments used in the remedial investigation and the record of decision process prior to the completion of the actual site remediation planning process.*

[**Hooper (Texas Tech University, in collaboration with the University of Washington)**]

4g.vi Clues to Methylmercury Levels in Freshwater Fish

Mercury enters the atmosphere from natural sources, such as volcanoes and forest fires; as a by-product of coal combustion to generate energy; from the use and disposal of mercury in products such as electric lights, computers, and blood pressure gauges; and from the production of chlorine and caustic soda. When mercury is deposited in lakes or waterways, bacteria convert some of it to a much more toxic form – methylmercury (MeHg). Methylmercury is recognized as one of the most hazardous environmental pollutants, and at high doses MeHg is a potent human neurotoxin that causes demyelination and delayed nerve conduction.

Fish accumulate metals throughout their lifetime and are a primary source of toxic metal exposure for humans. Mercury concentrations in fish have been related to metal burdens in their zooplankton prey, but potentially dangerous mercury concentrations have been found in fish from lakes that appear by other criteria to be “pristine”. Work conducted at Dartmouth College in 20 Northeast United States lakes showed that metal levels in the water alone are not very useful for predicting the level of several metals in fish across this gradient of lake types.

Biological Sciences Professor Carol Folt and graduate student Paul Pickhardt at Dartmouth College worked with a team of researchers at the University of Michigan in studies designed to identify mechanisms that enable toxic metals such as MeHg to move through aquatic food webs into fish. They tested the hypothesis that mercury inputs to lake food webs are impacted by the changes in algal biomass that follow nutrient additions to aquatic systems (primarily phosphorus and nitrogen).

Dr. Folt's research team simulated freshwater ecosystems in mesocosm stock tanks inoculated with phytoplankton and zooplankton. Six levels of nutrient loading were examined and the team added constant levels of unique stable isotopes of Hg to the experimental mesocosms. The researchers then measured the uptake of MeHg and inorganic mercury by algae and Daphnia, a common zooplankton herbivore known to be a major food for many planktivorous fish.

One achievement of Dr. Folt's team is that, primarily due to the efforts of Dr. Bjoern Klaue, they achieved spike detection limits 50-100 times lower than those of previous techniques. As a result, they are able to study the extremely low aqueous Hg concentrations that are typical of natural systems.

They made three significant discoveries:

- "Bloom dilution" occurs. The tanks with greater nutrient enrichment had greater algal biomass, so the same amount of mercury was distributed among a greater number of cells. This resulted in lower mercury per gram of algal material.
- The concentration of MeHg in Daphnia is related to the concentration of MeHg in the algal cells they ingest. Specifically, MeHg concentrations were consistently and significantly lower in Daphnia from the high nutrient, high initial algal biomass tanks compared to Daphnia from the low nutrient, low initial algal biomass tanks.
- Zooplankton that graze on algae preferentially accumulate MeHg relative to inorganic Hg. Bloom dilution of inorganic Hg concentrations in the algae had no measurable influence on the accumulation of inorganic Hg in Daphnia. To our knowledge, this is the first study to experimentally demonstrate the preferential accumulation of MeHg relative to inorganic Hg in grazing invertebrates feeding on a relatively intact phytoplankton assemblage.

These results suggest that *there is a link between the amount of algae in the water and the amount of mercury going up the food chain.*

On March 1, 2002, the United States Food and Drug Administration (USFDA) announced that it planned to schedule a meeting of its Foods Advisory Committee to review issues surrounding methylmercury in commercial seafood. This review will include a re-examination of USFDA's most recent Consumer Advisory for pregnant women and women of child-bearing age who may become pregnant. *The results of the Dartmouth study indicate that over the season in a lake, changes that cause algae to increase or decrease can also quickly produce changes in the amount of mercury that moves through the ecosystem. This finding is particularly important now as scientists and government officials*

try to determine how and when to measure mercury in order to issue more precise advisories about human consumption of fish.

[**Folt (Dartmouth College)**]

4g.vii Improving Ecological Risk Assessment: Development and Application of Methods to Determine the Bioavailability of Contaminants in Aquatic Sediments

Our ability to assess the potential risks of environmental contamination is often compromised by a poor understanding of the factors affecting the transport, fate, and availability of contaminants to both human and ecological receptors. Furthermore, even if we have adequate models describing the behavior of these contaminants, we often lack the analytical tools to obtain data assessing whether our current understanding of these mechanisms is sufficiently predictive of the presence or absence of unacceptable risks.

The objectives of Jim Shine's research at the Harvard University SPRP program are twofold:

- To improve our understanding of the mechanisms controlling the fate and effects of contaminants released to aquatic ecosystems
- To develop analytical tools that provide appropriate data to test the ability of these models to assess risk.

One part of this study is examining the partitioning and bioavailability of PAHs in aquatic sediments. Current models of PAH bioavailability in sediments assume equilibrium partitioning amongst three phases: the fraction freely dissolved in the porewater surrounding the particles, the fraction adsorbed by the organic carbon on the sediment particles themselves, and the fraction taken up by sediment dwelling organisms. Shine's research has shown that an additional phase, Black Carbon (a.k.a. soot carbon, char, or elemental carbon), has an extremely high sorption affinity for PAHs. In laboratory-based bioaccumulation studies using field collected sediments from various contaminated bays and estuaries, Shine's research team has demonstrated that although Black Carbon usually constitutes less than 1% of the sediment matrix, it has complexed greater than 90% of the PAHs. *The significance of this finding is that models that fail to account for the presence of Black Carbon likely over-estimate biological uptake (and subsequent human and ecological risks) by factors of up to 100.*

Shine and his colleagues have made an additional advance within this project by developing a sampling tool to assess the bioavailability of heavy metals in water and sediments. For heavy metals, it has been shown that the free metal ion (uncomplexed with particulate matter or dissolved organic matter) is the key determinant predicting metal distribution amongst different phases, including uptake by biological organisms. *They have developed and tested a passive sampling device, called the*

'Gellyfish', that can simultaneously determine the free metal ion concentration of multiple metals. The sampler, consisting of a small amount of a metal-binding resin held within polyacrylamide gel, is a significant improvement over current speciation methods that are labor intensive and can only determine speciation for one metal at a time. This sampler is a s*ignificant advance in the area of metal bioavailability research*, as it will allow one to conduct studies examining antagonistic and synergistic interactions of metal species in complex mixtures. *This will greatly enhance our ability to truly estimate the potential fate and effects of heavy metals in the environment.*

[**Shine (Harvard School of Public Health**)]

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4h. Innovative Spinoffs

By its very nature, basic research is not a linear process. It is impossible to predict all of the outcomes or foresee the eventual application of an investment. Due to the serendipitous nature of the research enterprise, investigators may start with a general concept of how their research could be applied, but then find that their results lead them in unanticipated directions.

Because the basic research supported by the Program tends to be use-oriented, you might think that it would be linear. In actuality, interesting spinoffs do occur. In some cases the spinoffs may have direct, but unanticipated applications to Superfund and in other cases the findings have broader application – but all relate to improving public health.

The SBRP staff recognizes that the most creative, cutting edge science results in innovative spinoffs; however, this has been a difficult area for us to track. Investigators tend to report to us findings that fit within the specific aims of their projects. Nonetheless, several investigators have brought to our attention new directions that have evolved from SBRP support. Some of these are presented below.

4h.i Development, Characterization, Optimization and Validation of a Recombinant Cell Bioassay System to Detect Dioxin and Related Chemicals

Halogenated aromatic hydrocarbons (HAHs), such as polychlorinated biphenyls (PCBs) and dibenzo-p-dioxins, dibenzofurans, are environmental contaminants that are present worldwide in wildlife, domestic animals, and human tissues as well as in food, water, and soil samples. Because of their ubiquitous distribution, resistance to degradation and high toxicity, these chemicals can have a significant impact on the health and well being of humans and animals. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, "dioxin"), the most potent HAH, can produce species and tissue-specific toxic and biological effects including reproductive dysfunction, birth defects, endocrine disruption, impaired immune function and cancer. Since an accurate measurement of dioxin-like HAHs in biological, environmental and food samples is critical for epidemiological and risk assessment analysis and for environmental monitoring, development of techniques for identification and quantitation of these compounds in various matrices is of critical importance. Although sophisticated cleanup and instrumental analysis procedures can separate, identify and quantitate individual HAHs, these procedures require expensive equipment and are also very costly and time-consuming. Thus, inexpensive and rapid screening assays capable of detecting and estimating the relative biological/toxic potency of complex mixtures of HAHs would greatly facilitate exposure and epidemiological analysis and screening for these chemicals in numerous matrices.

With support from the SBRP, Mike Denison at the University of California-Davis, has developed *a rapid and inexpensive recombinant cell bioassay system for the detection and relative quantitation of dioxin-like chemicals in sample extracts,* and it is amenable for the large scale screening analysis necessary for the above types of studies. This bioassay is based on the molecular mechanism of dioxin action and takes advantage of the ability of these chemicals to activate gene expression, specifically that of a firefly luciferase reporter gene that was stably integrated into human, rat and mouse cell lines. Exposure of this **C**hemically-**A**ctivated **LU**ciferase e**X**pression (CALUX) recombinant cell bioassay system to dioxins and dioxin-like chemicals results in induction of firefly luciferase in a time-, dose-, and AhR-dependent and chemical-specific manner. This assay is very sensitive (with a minimal detection limit of about 50-100 ppq TCDD) and validation studies reveal an excellent correlation between the amount of HAHs in a sample estimated by the CALUX bioassay and that determined by high resolution instrumental analysis. The CALUX bioassay has been optimized into a 96-well microplate format that will allow inexpensive, and rapid high-throughput screening analysis of relatively large numbers of samples/individuals in a relatively short time, which is critical for epidemiology and exposure assessment studies.

The CALUX bioassay is providing numerous researchers with a bioassay system to detect dioxin-like chemicals and related HAHs in small volumes of human tissue. *This assay has been used to assess the level of TCDD and related chemicals in blood samples from U.S. Vietnam Veterans (a collaborative study with the University of Texas) and women from San Francisco and Michigan who were exposed to these chemicals from consumption of contaminated fish from the San Francisco Bay and the Great Lakes.* The CALUX bioassay is also gaining widespread national and international use as an accepted method for detection of dioxins and related chemicals in extracts from a variety of matrices.

One major application that received little press, was *the key role that the CALUX bioassay played in identifying that a dioxin-like chemical was responsible for the Belgian food contamination crisis in 1999.* In this instance, the CALUX assay was used by Denison's collaborator in the Netherlands to demonstrate that contaminated chicken destined for human consumption contained relatively high concentrations of a dioxin-like PCBs. Given the successful application of our bioassay in this crisis, and recent double-blind validation studies, the CALUX bioassay is now being used as a screening bioassay for food products destined for human and livestock consumption by an agency of the Belgian government.

A United States biotechnology company has commercialized the CALUX assay and has recently licensed it to the United States Food and Drug Administration for similar purposes. In addition, the USEPA has expressed an interest in using the CALUX assay, as has representatives of the Government of Vietnam. The CALUX bioassay is also being used by a veterinary drug regulatory agency of the Korean government, to screen for dioxin-like chemicals in domestic animal feedstuffs. Overall,

Denison's group has provided the CALUX cells and vectors to more than 55 research labs worldwide. These systems are now being used for a wide variety of purposes, including detection of dioxins in a wide variety of environmental, biological and food samples. and as a possible screening assay for evaluation of remediation technologies.

[**Denison (University of California – Davis)**]

4h.ii A Novel Intervention Strategy to Reduce Exposure to Contaminants in Food and Feed

Molds and their chemical products can be beneficial as well as harmful to humans and animals alike. The Chinese wrote about the effects of poisons from molds almost five thousand years ago, yet scientists are still seeking practical ways to deal with and reduce our exposure to these elusive chemicals. Tim Phillips, an SBRP-supported researcher at Texas A&M University, has developed a novel intervention strategy using a clay mineral in the diet to reduce aflatoxin exposure from food and feed.

Potent mold-derived chemicals known as "mycotoxins" can be found in nearly all mold-contaminated food sources and may be harmful if ingested in high enough quantities, or over a long enough period of time. Fortunately, the toxicity of these compounds is dose-related, and their levels in foods in the United States are typically low to non-detectable. However, during extended periods of drought, or with poor storage conditions, the production of certain harmful chemicals can be significantly higher and result in contaminated products that present a potential health risk.

Of the mold-derived chemicals frequently found as contaminants of food, those known as aflatoxins can be acutely toxic, suppress the immune system and have been shown to cause cancer based on both laboratory and epidemiological studies. Exposure to aflatoxin in less than lethal doses may cause vaccinations in farm animals to fail, potentially resulting in greater incidence of infectious diseases, and may cause slower growth. The scope of the aflatoxin problem is global, but the aflatoxins especially threaten over-populated developing countries and rural communities with either limited food-safety resources, high rates of infectious disease, or frequent consumption of highly contaminated food.

To combat aflatoxins in animal feed, Phillips is working with a clay product called NovaSil (an anticaking additive) as an additive to animal feed. *NovaSil can bind tightly and preferentially to aflatoxins in the digestive tract, and safely protect animals from these poisons, thereby preventing their uptake by the blood and target organs and reducing their toxicity and cancer-causing effects.* The molecular mechanism is thought to involve reaction of the dicarbonyl system in aflatoxin at multiple sites on the surfaces of NovaSil, especially those sites associated with the interlayer or gallery region.

Phillip's patent (for the enterosorption of aflatoxin with NovaSil clay) is currently being applied on a worldwide basis as an animal feed additive – *NovaSil is routinely added to the diets of farm animals in the United States, Latin America, Asia, Africa, and Europe. Estimates are that the Phillips technology is now being used in ten percent of animal feeds worldwide.*

Phillips is optimistic that this same technology, which is culturally acceptable in developing countries may one day be applicable and sustainable for the protection of human foods decreasing human exposure to and suffering from aflatoxins. He summarizes his future plans as follows: "My long term goals are to further confirm the safety of NovaSil prior to human studies, followed by short-term human feasibility studies, where the smallest effective dose of NovaSil clay will be delivered by mouth using capsules or flavored slures before meals. In these initial studies, biomarkers of aflatoxin exposure in urine and blood will be measured to determine the effects of clay treatment."

[**Phillips (Texas A&M University)**]

4h.iii Detecting and Analyzing Patterns in Spatial Data

Perceived patterns in spatial distributions are among the most provocative clues to environmental etiologies. Incomplete survey data or maps made from easily obtained routine vital events registries often appear to reveal local disease clusters. Public interest and demand for such intuitively accessible data is strong. On the other hand, epidemiologists are often uneasy about the methodological limitations of these kinds of data and many believe follow-up is unproductive. There is therefore renewed interest in understanding the kinds of biases inherent in such data and developing means to cope with them. Disease "hot spots" may be due to clustering of a known risk factor (e.g., a cluster of lung cancer might be caused by an excess of smokers in the area). Diseases like cancer are typically caused by exposure years earlier (latency); where one lives at time of diagnosis is then less important than where one lived in the past.

Addressing these problems requires taking into account known risk factors for a disease as well as residential history. Detailed information of this type is rarely available from routinely collected data. David Ozonoff at Boston University conducting a complex data analysis project, applying geographically coded cancer data from Cape Cod (from his earlier SBRP-funded research) and reproductive/developmental effects data (from Ann Aschengrau's current SBRP-funded project) to provide such a resource.

New methodologies are needed to construct maps free of the earlier problems. *Ozonoff and his colleagues have developed methods that combine current software for manipulating geographic data (GIS) with advanced statistical methods to produce disease maps useful for investigating the role of environmental exposures in disease risk.* The research team employs generalized additive models (GAMs), a type

of regression that combines smoothing with the ability to analyze data from population based case-control studies. Smoothing on latitude and longitude produces maps that estimate the rates of disease relative to the whole study area. By taking into account known risk factors (e.g., age, smoking), *Ozonoff can estimate the residual risk due to other potential determinants of disease, including environmental exposures.* These methods also provide statistical tests for the importance of location in the model as well as the identification of local "hot" and "cold" spots. By overlaying maps of disease and environmental exposures, new causal hypotheses may be suggested.

After testing the method using synthetic data, Ozonoff's group investigated the association between residential history and colorectal, lung and breast cancer on Upper Cape Cod, Massachusetts using studies of cancer with extensive data on residential history. Little or no change was seen in the maps after adjusting for potential confounders, indicating that the individual risk factors they analyzed did not account for the spatial patterns of disease. In other words, clustering of disease in this area was not caused by known risk factors. In preliminary results, no associations between colorectal cancer and location were observed.

Assuming 15 years of latency, lung cancer was significantly elevated in an area just northeast of the Massachusetts Military Reservation (MMR), a Superfund site. Breast cancer "hot spots" tended to increase in magnitude as latency is increased. *Significant "hotspots" were located near two known pollution plumes and the MMR.* One hot spot was near a contaminated pond whose wildlife are being studied by another project in another Boston University SBRP-funded project. While additional work is needed to confirm these spatial patterns, Ozonoff's maps suggest further investigation of the pollution plumes and analyses based on detailed exposure modeling.

Collection of detailed data on individuals is expensive and time-consuming. Access to aggregated data (e.g., disease rates by town, fraction of the public on public water supply) is usually easier and cheaper. Unfortunately, studies using aggregated (group-level) data can be subject to substantial bias. In another part of his research, Ozonoff has been working to better understand and quantify these biases and has discovered that studies that use partially aggregated data, which is fairly common in environmental epidemiology, can also be subject to some of these problems.

[**Ozonoff** (**Boston University**)]

4h.iv A Cellular Biosensor to Detect Chlorocatechols

Catechols, including chlorocatechols, are introduced into the environment by both industrial and natural sources. Because these compounds are resistant to many standard wastewater treatment processes, they may be discharged in the effluents of paper, rubber, dyes, plastics, pharmaceuticals, and cosmetics manufacturing facilities and accumulate in lake and river sediments. More

importantly, catechols are produced naturally in the environment as the by-product of microbial degradation of chlorinated aromatic compounds including polychlorinated biphenyls (PCBs). The catechol by-products are more toxic than the parent compounds. Catechol and chlorocatechols have been characterized as strong irritants to the eyes, skin, and respiratory tract and have been proven to cause DNA damage.

Sylvia Daunert leads a team of scientists at the University of Kentucky (UK) who have developed a cellular biosensor system to identify and quantify 3-chlorocatechol and 4-chlorocatechol, both toxic breakdown products of PCBs. A biosensor is a biological monitor that recognizes a chemical or physical change and produces a measurable signal in response to the environmental change. Cellular biosensors can be designed for high sensitivity and selectivity, but the greatest advantage of cellular systems is they are capable of measuring the bioavailability of the compound being studied. Cellular biosensor systems operate as follows:

1. The compound being studied passes through the cell membrane.
2. The compound binds to a regulatory protein.
3. The regulatory protein activates a promoter.
4. The promoter activates the reporter gene.
5. Translation of the reporter gene produces a reporter protein.
6. Upon addition of an external substrate, the reporter protein produces a measurable signal.

To detect 3-chlorocatechol and 4-chlorocatechol, the UK researchers applied genetic engineering techniques to design and develop a cellular biosensor system using the bacteria *Pseudomonas putida*. These bacteria can use chlorocatechols as a carbon source, biodegrading chlorocatechols via a degradative pathway regulated by the ClcR protein. The UK researchers introduced a plasmid that contains the lacZ gene into *P. putida*. The lacZ gene is also controlled by the ClcR protein and encodes for b-galactosidase.

The researchers verified that the system is extremely specific. They exposed the biosensor system to a series of organic compounds (catechols, chlorophenols, biphenyl) that are structurally related to chlorocatechols and might interfere with the biosensor system. No appreciable levels of b-galactosidase were produced. The UK scientists have also demonstrated that the system is capable of distinguishing 3-chlorocatechol from 4-chlorocatechol.

By developing this sensitive, selective system, the UK scientists have provided hazardous waste site remediators with a valuable tool for directly identifying and quantifying chlorocatechol compounds in the complex mixtures commonly found at Superfund sites. This cellular biosensor system may be useful for several practical applications:

1. Assessment of environmental contamination - the system can be used to detect low concentrations of chlorocatechols in soil, sediment, water, and biological samples.

2. Evaluation of remediation efforts - it is important to ensure that remediation systems designed to clean up PCBs do not leave the site contaminated with chlorocatechols.
3. Exposure studies - chlorocatechols have been found in the urine of individuals exposed to chlorobenzene and therefore can serve as biomarkers of exposure.

This biosensor system is a tailor-made probe to directly monitor the level of chlorocatechols in soil, sediment, and water samples at Superfund sites. Because it does not require expensive equipment or extensive pretreatment of environmental samples, the UK biosensor system is simpler and more economical than standard methods. The UK scientists are working to develop additional biosensor systems. By using different reporter proteins that emit fluorescence or bioluminescence at different wavelengths, they are developing array sensing systems for a variety of environmental pollutants. Analysis of the color of the light generated by the bacteria will allow for identification of the pollutant(s) present in a particular environment. This would provide tools for *in situ* monitoring of multiple contaminants at Superfund sites.

[**Daunert (University of Kentucky)**]

4h.v Development and Implementation of Immunoassays for Human and Environmental Monitoring

One of the major impacts of the UC Davis SBRP is just now being realized. This is the largest and most comprehensive human health study ever done on worker exposure to the herbicide paraquat. The work was initiated many years ago from the Epidemiology core, the Immunoassay project and the Analytical Core. Paraquat is an interesting compound. It is an environmental benign (or green) compound, which is a broad spectrum herbicide. Because of its broad spectrum, low weed resistance and low cost it is seen as an essential tool in many agricultural ecosystems. This is particularly true in developing countries. On the downside *paraquat has caused more human deaths than all other pesticides combined.* Not only does it have high acute toxicity but paraquat exposure has been associated with a variety of chronic problems.

Key to this study was the *development of sensitive and specific immunoassays to detect paraquat exposure based on urinary levels of paraquat by researchers in Bruce Hammock's laboratory.* They have run hundreds of urine samples of workers on coffee and banana plantations in Costa Rica and associated the exposure with the worker's job (mixer loader, sprayer, irrigator). These workers have had a complete health history taken and a full physical including pulmonary function. *With paraquat currently undergoing re-registration in many countries and being reconsidered for sale in the United States, the outcome of our studies will be of great significance.*

The UC Davis SBRP work in the immunoassay project also provided preliminary data to secure a $165,000 contract from the California State Water Resources Control Board to develop a toxicity

identification evaluation (TIE) protocol to identify pyrethroids. Dormant agricultural sprays are a major tool and critical in reducing the total environmental burden of pesticides. However, the procedure often results in chemical run off into aquifers. This study will evaluate and help to reduce fish kills from run off.

In addition, ongoing SBRP-funded research in Hammock's lab provided preliminary data to secure a $775,000 grant from the Department of Defense to conduct a human exposure to study permethrin and develop an analysis method for a urinary biomarker of exposure to this compound. This work will provide the first human metabolism study of relevant doses of pyrethroids and has a direct impact on theories regarding the Gulf War Syndrome. *Since pyrethroids will be used to control adult mosquitoes that may spread the West Nile Virus, this knowledge comes at a critical time to impact human health.*

[**Hammock (University of California – Davis)**]

4h.vi Identifying the Mechanism by which *B. Anthracis*, the Causative Agent of Anthrax, Escapes Detection by the Innate Immune System

Work carried out in Dr. Karin's laboratory over the past two years has focused on the use of genetically altered strains of mice to uncover mechanisms that determine sensitivity and susceptibility to environmental toxins and stress. This work has played a central role in defining the actions of stress activated protein kinases, such as the mitogen activated protein (MAP) kinases JNK, p38 and the IkB kinase (IKK) complex. The most notable advances have been based on the generation of JNK1 and JNK2-deficient mice and a conditional IKKb knockout mouse. These animals are being used not only in Dr. Karin's laboratory to examine the effects of oxidative stress induced by environmental exposure, but by other groups in the SBRP that have found these MAPK deficient animals to be of value in studying biological responses relevant to their projects. An example of the unforeseen importance of this approach was realized when Dr. Karin adapted an approach to investigate the importance of these MAPK deficient animals to understanding the toxicity initiated by exposure to anthrax.

It has been known for quite some time that during inhalation anthrax, the spores are taken up by alveolar macrophages, which then transport them to lymph nodes where they germinate and are released to the circulation. One of the unique features of anthrax infections is that they progress without being detected by the innate immune system until the bacteria reach a very high titer. Karin found that the lack of detection by the innate immune system is most likely due to the ability of the anthrax lethal toxin (LT) to enter macrophages where it inhibits the activation of most MAPKs, of which the most important is p38. Once macrophages lacking p38 activity are exposed to lipoteichoic

acid, a component of the Gram positive cell wall (*B. anthracis* is a Gram positive bacterium), they undergo very rapid apoptosis and fail to secrete chemokines and cytokines, which otherwise alert components of the innate immune system to the presence of the pathogen. Indeed, Dr. Karin discovered that exposure of macrophages to the lethal toxin of *Bacillus anthracis* followed by exposure to endotoxin, which is produced by Gram negative bacteria, or lipotechoic acid, which is produced by Gram positive bacteria, results in rapid apoptosis instead of activation and cytokine secretion. *Further experiments revealed that this is the likely mechanism by which* B. anthracis*, the causitive agent of anthrax, is evading detection by the innate immune system and explains why anthrax infections go undetected until the bacterium reaches an extremely high titer in the circulation.*

With the current concerns in America regarding bio-terrorism, this work is highly significant and may lead in the future to the development of chemical inhibitors that may be effective in blocking the biological actions of anthrax exposure.

[**Karin (University of California – San Diego)**]

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4i. Contributions of Outreach Programs

The decisions made regarding the disposition of hazardous waste sites are a result of a long, complex process involving risk assessors, site managers and affected communities. Frequently the information available to the communities participating in this process is extremely technical, fraught with scientific uncertainties, and sometimes contains conflicting messages. The USEPA and ATSDR both face significant challenges in addressing community needs when the available data and knowledge are incomplete.

While we recognize that the USEPA and ATSDR are on the front line in responding to community needs, we also realize that SBRP grantees can serve as important and independent resources to these agencies and the communities as they struggle to address the science and site-specific issues. At one level we expect each grantee to work directly with regional USEPA, ATSDR and state offices; however, we also encourage each grantee to include "outreach" in its overall research plan. The intent is that through outreach activities the research emanating from the SBRP will increase the communities' understanding of environmental health. Conversely, and of equal importance, involvement with the community serves to inform the researcher of the public's most significant concerns. As you will read on the following pages, current activities range from education to improving public awareness to responding to public and community concerns.

4i.i SBRP Partnership with the Children's Environmental Health Network

In 1992 the SBRP joined in supporting the Children's Environmental Health Network (CEHN), an organization that led a national effort in advocating children's environmental health. The CEHN identified three major areas of need in order to better protect children from environmental exposures – the need for:

- a national policy
- educational programs for health care professionals
- a national research agenda.

The SBRP worked closely with the CEHN in the early 1990's to set a research agenda in this area. This was accomplished primarily through a series of successful workshops and conferences – the first of these was held in 1993 at the NIEHS. In 1997, one of CEHN's goals was realized when President Clinton issued the Presidential Executive Order "Protection of Children from Environmental Health and Safety Risks". This order gave children's environmental health research national recognition and validated the SBRP's initiative in this area.

The SBRP continues to support research that focuses on children in programs that:

- Compare the effects of exposures in children to those observed in adults
- Investigate the effects of prenatal or *in utero* exposures
- Provide education to parents and expectant parents to help them take steps to protect their families from avoidable exposure to environmental contaminants.

4i.ii The "Environmental Health Food Safety Scenario"

In July 2002, nearly one hundred minority students from the Boston area, ages 14-17, participated in a three hour Environmental Health Food Safety Scenario at the Boston University Medical Center. Organized by members of the SBRP Outreach Core at the Boston University School of Public Health (SPH) and the Boston Area Health Education Center (BAHEC), the goals of the exercise were to:

- Illustrate "public health in action"
- Identify who is involved in, and affected by, public health
- Introduce dioxin and PCBs and their health effects to students
- Teach about food systems, food industry and food safety

The scenario began with BAHEC students at their annual summer picnic where they ate "a lot of chicken," The following morning the Boston Globe reports:

> "Widespread chicken contamination… What appears to be a mass poisoning has affected poultry farms across the states of Alabama, Georgia and North Carolina, with an estimated loss of over 100,000 chickens. According to a grower who requested anonymity, the chickens acted very strangely for the last several days and 'wouldn't go near their feed.' Fred Pucker, CEO of PuckerUp Poultry Inc., which hires out the farms where the poisoning occurred, refused to be interviewed. The Alabama and North Carolina State Departments of Public Health are investigating the outbreak to determine the risk this poisoning may pose to human health. Poultry is a major industry in the South."

Students were asked: What made the chickens sick? Does this affect humans?

Should we, in Boston, be concerned about chickens in North Carolina, Alabama and Georgia? Students were unsure; some laughed, and some were thoughtful. They were then asked to switch hats and become investigative journalists working for the Boston Globe. The late-breaking news was that according to the NC DPH, the chicken fat is contaminated with dioxin and PCBs.

Students in groups of eight had seven minutes to interview each of the following characters:

- Fred Pucker, CEO of PuckerUp Poultry
- an Alabama DPH official
- a MA DPH official

- a Public Health Scientist with expertise on dioxin (played by Dr. David Sherr, an SBRP-funded researcher)
- a concerned community person from Kingston, Alabama (played by SBRP graduate student Veronica Vieira)

Students came back as a larger group to report their findings. They discovered early on that the information provided by different experts was often conflicting, and they described the factors that contributed to their trust in some "experts" and their distrust in others.

The final part of the exercise consisted of two BU SPH faculty (David Ozonoff and Tom Webster, both BU SBRP researchers) sitting on a panel with an "Oprah Winfrey"-like moderator soliciting questions from the students. The seriousness with which the students posed their questions to the panel provoked one of the organizers to remind students that this was a fictitious scenario. The students wanted to know why dioxin persists in the environment if it makes people sick, how they could be sure their food supply is safe, whom in the government they should speak with, and how they can participate in public health decision making.

The Boston University Outreach Core succeeded in its goal to contribute to the education of high school students and encourage and interest them in careers in environmental health.

[**Scammell/Ozonoff (Boston University)**]

4i.iii Environmental Health Education for Nurses

In the United States, there is a major deficit with respect to environmental health education and the health impacts of environmental toxins and hazardous waste practices in the education of nurses at the undergraduate and graduate levels.

Stephanie Chalupka, RN, EdD, a Visiting Scholar in the Boston University SBRP Outreach Program, has developed *The Core Curriculum in Environmental Health*. The contents address the competencies specified by the National League for Nursing. The development of the program was funded by the ATSDR and the American Association of Occupational Health Nurses (AAOHN). The Harvard School of Public Health SBRP provided content-related resources for the curriculum and consultation time with Dr. Susan Korrick, an SBRP researcher. The goal of the curriculum and of the outreach to nurses is to increase the capacity for environmental health nursing in the United States and basically to build a cadre of nurses who include environmental health nursing in their practice.

The text covers topics including:

- The nature and scope of environmental hazards
- Distribution of toxic agents in the environment
- Exposure pathways
- Hazard classification
- Vulnerable populations with a special emphasis on pediatrics
- Toxic threats to child development
- Risk assessment and communication
- Methods for obtaining an environmental exposure history
- Priority research areas
- Advocacy for environmental health
- Role of occupational and environmental health nurses in promoting environmental health.

The curriculum is now in the dissemination phase. Twelve "train-the-trainer" workshops for nursing faculty will take place over the next year. Workshops or additional conferences already accomplished in 2002 include "The Impact of the Environment on Health," at UMASS Lowell; "Environmental Health – Nurses Making the Connection for Health and Well Being," American Nurses Association, Pre-conference Workshop, Philadelphia; "Core Curriculum in Environmental Health," University of Illinois at Chicago's Great Lakes Center for Health and the Environment, Chicago; and "Nursing, Health, and the Environment – Strengthening the Relationship to Improve the Health of Vermonters," Burlington, VT.

In the New England region, the Burlington, Vermont Conference "Nursing, Health, and the Environment" sponsored by the Vermont Department of Health, Tufts University, and the Harvard School of Public Health, laid down the framework that will enable the 12 Environmental Health Specialists who service all 14 counties in Vermont to address environmental health issues at the individual community level and potentially to reach the entire population of 600,000 people. Coordinated by another HSPH Visiting Scholar, Eileen Girling, Director of Community Health Nursing at the Vermont Department of Health, this "EH Specialist" structure provides a direct link between communities and the Vermont Department of Health with respect to issues such as clean drinking water, landfills, and toxic wastes.

To continue to enhance environmental health nursing, the Vermont Department of Health has established collaborative efforts with the University of Vermont Nursing program (nursing education), and the Harvard School of Public Health SBRP (GIS project assistance).

[**Backus/Monson (Harvard School of Public Health)**]

4i.iv Teacher Training in Environmental Health

The goal of the University of Florida Outreach Core is to disseminate knowledge about environmental health research related to Superfund chemicals to other scientists and the general public. In 2002, the Outreach Core staff expanded its existing teacher education program to include middle and high school teachers selected from schools near Superfund sites in Florida and Louisiana.

Following the success of the pilot program conducted with 15 teachers in 2001, there were more highly qualified applicants for the program than expected, and the number selected was increased to 20. The program was structured to include an intensive one week hands-on course held on campus at the University of Florida, which featured four SBRP-funded studies related to:

- ecological effects of estrogenic chemicals
- effects of Superfund chemicals on reproduction, fertility and women's health
- remediation of contaminated sites

The program also featured training from staff in the Analytical Chemistry Core and the Histopathology Core, and a session on risk assessment from the outreach core. The research problems being addressed were linked to the Superfund sites near the teachers, and the teachers were asked to research this aspect.

Following the on-campus portion of the program, the teachers were expected to:

- Develop and implement an action plan for including what they had learned about environmental toxicology in their lessons
- Present their results to the SBRP faculty back in Gainesville

The teacher participants were highly enthusiastic about the opportunity afforded to them to learn more about research in toxicology. The participants have evaluated the program very well. Significantly, the impact of this program extends beyond the participants as these 20 teachers have contact with over 100 students each that they teach regularly – for a total of well over 2000 people served by this program.

[**Koroloy (University of Florida)**]

4i.v Regional Workbench

A major part of the University of California-San Diego (UC-SD) Outreach program focuses on building an innovative *Regional Workbench Consortium* (RWBC), **http://regionalworkbench.org**. The RWBC is a collaborative network of university and community-based partners located throughout the San Diego-Tijuana city-region. The outreach mission is to link SBRP science and

technology with environmental policy, planning and education. The RWBC is thus *creating the technical and social conditions necessary to integrate regional data, information and knowledge.*

The RWBC focuses on specific projects that demonstrate multidisciplinary and multiagency collaborations in a partnership-based approach. For instance, Julian Schroeder (a UC-SD SBRP-funded researcher), along with several of our high school outreach students, has been analyzing water samples in the San Diego region collected by the Baykeeper – a local non-governmental organization that has an extensive Border Coastal Water Monitoring Project. The UC-SD Outreach Core has begun to geo-reference the Baykeeper's water toxicants data in an on-line Geographic Information Systems (GIS) and interactive mapping system. *This makes the results more accessible to the public and decision-makers, and it provides SBRP scientists with meaningful context for their work.*

The UC-SD Outreach Core works closely with a range of public and private sector organizations including the San Diego Association of Governments, the San Diego Regional Water Quality Control Board, the Industrial Environmental Association, TELESIS Corporation, and the Association of Collegiate Schools of Planning. To link SBRP science to the needs of such agencies, the Outreach Core is developing scientific visualization tools (merging GIS and 3D imagery), multimedia narratives, user-friendly search engines, data mining portals, and quality of life indicators.

[**Pezzoli (University of California – San Diego)**]

4i.vi SBRP Outreach Core and Community Partners Implement Preventive Intervention Programs in Children's Environmental Health

Duke University's Superfund Center Outreach Core is successfully partnering with community-based organizations in Durham County, North Carolina to implement preventive intervention programs in children's environmental health. In conversations with community-based organizations regarding their sense of community needs and interests, it became apparent that childhood lead poisoning was a special concern. Multiple Duke SBRP investigators have expertise in this Superfund chemical, including Di Giulio, Freedman, Vasudevan, and Miranda.

Duke's in-house SBRP GIS expertise allowed for the development of lead exposure risk priority maps. Maps drawn from a GIS project were developed for Durham County, North Carolina and used spatial analysis of county tax assessor, U.S. Census, and North Carolina blood lead screening data to categorize lead risk levels at the individual tax parcel unit level. The maps predict the most likely parcels to contain lead paint hazards. Areas that are least likely to contain lead paint hazards were also identified. Compared to South and Northeast Durham, the map predicts that Central Durham has a heavier concentration of higher risk parcels.

Durham Congregations, Associations, and Neighborhoods (Durham CAN) and the Durham Affordable Housing Coalition (DAHC), have been using this model as the basis for targeting communities in an ongoing effort to increase blood lead screening among high risk children. Outreach Core staff have partnered with Durham CAN and DAHC in presentations to PTAs, childcare centers, and churches. Other maps zoom in on specific neighborhoods and are printed in large-scale format for use in community presentations. The population of children screened through this effort is exhibiting a 10% prevalence rate of elevated blood lead levels, well above Durham County's average prevalence of just over 3%.

The broader expertise of Duke's SBRP investigators also allowed the Outreach Core to develop a summary of the most recent research on neurological effects associated with low level lead exposure in response to a joint request from the Durham County Health Department, a series of community-based groups (Durham CAN, the Durham Affordable Housing Coalition, PEACH), and local health care providers. The request was received through the Outreach Core staff's participation in the Durham County Lead Action Team. This document is being mailed to every pediatrician and community and family health practitioner in Durham and Orange Counties; it has also been used as the basis for Continuing Medical Education Training in other North Carolina counties.

These successful interactions focused on lead have helped to better establish the expertise and availability of Duke's SBRP generally and the Outreach Core more specifically. The Outreach Core is now engaged in discussions with community groups and local health departments to use SBRP expertise to help identify groundwater contamination risk associated with Superfund sites, CERCLIS sites, underground storage tanks, and TRI-reported discharges.

[**Miranda/Di Giulio (Duke University)**]

4i.vii Health Education to Reduce Exposure to Environmental Contaminants

The University of Washington (UW) SBRP Outreach Core has established partnerships with communities at two contaminated sites in the Puget Sound area. The Lower Duwamish Waterway is a Superfund Site, jointly managed by Washington State Department of Ecology (DOE) and USEPA Region X. It was added to the National Priorities List in September 2000, and remedial action is in progress. The UW Outreach Core staff is working with the Washington Department of Health (DOH) and the International District Housing Alliance (IDHA) to develop and implement innovative hands-on activities to help those who catch and eat fish from the Lower Duwamish Waterway reduce their exposure to environmental contaminants.

Youth from IDHA's after school program have been trained in various environmental health and justice topics in partnership with Outreach Core staff. Food safety and seafood consumption

have been the special focus. IDHA youth and DOH staff demonstrate fish cleaning and cooking techniques at community meal sites, where elderly Asian Pacific Islander (API) community members gather. *This intergenerational approach to health education has proven effective in the API community.* The fish cleaning sessions at the senior meal sites will begin in Spring 2003. The activity will reinforce that seafood consumption is part of a healthy diet, but that there are ways to reduce chemical exposure associated with eating Duwamish River fish and shellfish.

In addition to work on the Lower Duwamish site, the UW Outreach staff is assisting the Snohomish Health District with a new community health advisory for residents near the Everett Smelter Site. The smelter site is under the jurisdiction of DOE. Remedial action is in progress and contaminated soil is being removed from the most polluted areas. Residents in the neighborhood are homeowners, renters and public housing residents. Summaries of the health advisory materials have been translated into languages other than English. The Outreach Core will work with the Snohomish Health District and the Everett Community Coalition to produce additional materials regarding site contamination, focusing on health and safety precautions for children.

[**Burbacher (University of Washington)**]

4i.viii Facilitating Community Participation in Superfund Decisions: The Elizabeth Mine

One impediment to community participation in decision-making related to new Superfund sites is the inaccessibility of documents containing necessary information. Obstacles include the location and hours of document repositories, costs of photocopying, and the timely release and distribution of pertinent data such as scientific reports by consultants. *In partnership with community members in Strafford, Vermont, Dartmouth's Toxic Metals Research Program and Center for Environmental Health Sciences facilitated development of two web sites to help citizens' efforts to sort out decisions related to the listing and cleanup of the Elizabeth Mine – a new Superfund site in Vermont.*

The Dartmouth-led team developed the first website with students and teachers from the Newton School in Strafford, Vermont, who spent a month investigating and reporting on the scientific, historic and cultural aspects of the former copper mine. The community's logistical difficulties in sharing documents prompted development of the second web site: a document-sharing mechanism for citizens. Working with the mediator for the Elizabeth Mine Citizens Advisory Group, several other Advisory Group members and a Dartmouth undergraduate, the Outreach staff developed a process for identifying, compiling and editing information important to different stakeholders. In addition to scientific and technical reports from the USEPA and consultants, this included background on the site's historical and cultural relevance.

The project illustrates that websites developed in partnership with communities can provide stakeholders more control over the scope and accuracy of information necessary for decision-making. In addition, scientific information on a stakeholder-designed website can appear within its social, cultural and historic context. The project is also a model for serving the diverse information needs of a community dealing with a new Superfund site. Such a "community" is often several communities, and the needs of those communities change over time.

Outreach staff were invited to present this community collaboration as a case study at the May 2002 conference of the United States Institute for Environmental Conflict Resolution.

[**Serrell/Hamilton (Dartmouth College)**]

4i.ix Community Based Prevention and Intervention Project: Community-Based Metal Exposure in Child Development and Hearing

Dr. Robert Wright and Dr. Howard Hu are conducting prevention and intervention projects to address community concerns identified through biannual focus groups arranged by and held in conjunction with Local Environmental Action Demanded (L.E.A.D.), a local community group.

As an example of community feedback/empowerment, at the last focus group the Harvard researchers addressed which toxins are of primary concern to the community. Tar Creek is a rural area in Oklahoma, and agriculture is a primary industry. Surprisingly, one of the toxins identified was unrelated to metals from mining chat or to pesticides from agriculture. Benzene was found to be a concern for this community. A benzene plume was recently identified beneath the area, which is moving toward Grand Lake, a primary recreation and water supply site. This concern was only identified because this is a community based research project in which the community is a partner. Future studies to address exposure to benzene are being planned.

At this focus group Wright and Hu also raised the issue of nutrition as a means of reducing individual exposure to metals. Both calcium and iron deficiency are associated with increased lead absorption. With L.E.A.D, they are planning a new campaign targeting pregnant mothers to assess dietary intake of each nutrient and increase calcium and iron in their diets when appropriate. They also plan to consider cultural differences in foods consumed, with an emphasis on Native American diets. This work is significant because it is aimed at all pregnant women, not just those who may be exposed to environmental toxins.

[**Wright/Hu (Harvard School of Public Health)**]

4i.x Human Health Assessment Survey

While serving as a Visiting Scholar in Harvard's Outreach Core, Steven Dickens of River Network worked to develop human health assessment tools that could help communities investigate, and thus better understand, the human health impacts of environmental contamination, particularly involving water. As part of the first phase of the Human Health Assessment Survey project, River Network conducted a nation-wide needs assessment to identify communities that could benefit from these tools and to create a database that would enable these communities to network with one another.

The *Healthy Waters, Healthy Communities* project that Mr. Dickens directs has published the recently completed Needs Assessment on the River Network website **http://www.rivernetwork.org/health**.

Data have come in from over 142 communities nationwide. Communities with suspected environmental health problems reported bacterial contamination as the most frequent contaminant of concern (26% of respondents) followed by unspecified industrial chemicals (17%), heavy metals (16%), PCBs (10%), and pesticides (7%). Fifty-two percent of the respondents characterized this contamination as serious or very serious.

Although many of the communities cited discrete health problems such as cancer, gastro-intestinal problems, respiratory problems, birth defects, immune system problems, and urinary tract problems, only 36% of the respondents reported being certain or somewhat certain that there was a relationship between the water and the health impact.

When asked what type of help communities needed, the community respondents cited help with monitoring, health surveys, characterization of the discharge into the water, identification of the hazard, and help with web pages for communication as the top five needs.

In a pilot community project, River Network helped the environmental staff at a northern New Mexico Pueblo monitor their rivers for bacteria, and conduct a subsequent health survey. Amongst other findings, there was a statistically significant relationship between women who reported drinking from irrigation ditches (adjacent to the rivers) and gastrointestinal illness. Most surprising was the number of people who reported regularly drinking untreated river water, and thus the degree of exposure and potential health risk. These findings necessitated a recommendation for regular monitoring of the river for bacteria and for conducting a community-wide education campaign about the use of the water for drinking, and land-use practices that can impact the quality of water.

The development of this human health assessment tool moves River Network into a new phase of their work with river monitoring. It brings the component of human health to the fore and enables communities to focus not only on the contamination and condition of their local river but also on the human impact of that contamination. With better definitions of the problem and the health consequences, more appropriate monitoring and cleanup modes can be established; more appropriate

resources can be mustered to solve the problem; and better decision-making at the community level can be expected. Better definition of the problem in terms of human health and contaminant identification will also enable our SBRP faculty to provide more specific assistance to River Network especially in the area of heavy metals and PCBs if requested.

As a starting point this human health assessment tool will be the foundation for more detailed work with groups in two communities this year. River Network has already begun work in concert with the Southwest Organizing Project to help a community group in a small ethnically and economically diverse town in central New Mexico. Other communities and groups that River Network is considering working with this year include tribes in California, Maine and New York states, and an active river group in Oregon.

The initial web-based human health community needs assessment will be made into an ongoing registry where potentially hundreds of new communities around the country can log their problems and network with others, including service providers. River Network will continue to use that registry to identify communities most appropriate to work with. The SBRP stands to learn a lot from River Network about communities and their needs, and faculty from the SBRP will continue to serve on the Healthy Waters/Healthy Communities Advisory Committee and to provide resources as appropriate to River Network.

[**Backus/Monson (Harvard School of Public Health)**]

4i.xi The River Project

One of the main highlights for the Mount Sinai School of Medicine Outreach Core has been its partnership with The River Project, a community-based, research organization focused on the protection and restoration of Hudson River wildlife through research, hands-on programs and urban ecology projects.

Through this, the Outreach program has provided a forum for SBRP researchers to share information with residents of the Hudson River region, and has provided 10 scholarships for students doing research on Hudson River contaminants.

One such research project, performed by River Project interns and headed by Mount Sinai Summer Research Fellow Astrid Huertas Hernandez, was presented at the SBRP Annual Meeting held in Tucson in November. The project focused on the effects of pollutants like bacteria, organic matter, floating debris and other toxic substances coming from combined sewer overflows in the river. The study aimed to design technology that could be transferred to the field so that community

organizations near the Hudson could quantify the effects of sewage on bacterial concentrations, and map the transport and fate of the sewage in a near-shore area.

A simple tracking and mapping system was designed and will be used in future research collaboration between The River Project, Mount Sinai and the Borough of Manhattan Community College. A policy implication of this project is to help influence how the New York City Department of Environmental Conservation measures bacteria levels for the purpose of making decisions on the potential for recreation along the waterfront.

Also, this year, through the Community Outreach Core's partnership with The River Project, Mount Sinai Superfund Researcher Dr. Anne Golden presented her study of Urban Anglers to a group of 50 local residents as part of a summer-long workshop series presented by The River Project and sponsored by the Outreach Core. The workshops serve as an opportunity for researchers to share their work with communities affected by Hudson River contamination.

[**Claudio** (**Mount Sinai School of Medicine**)]

4i.xii Bolstering Limited Nonprofit Resources with Research Support

The NC Conservation Network (ConNet), a statewide coalition of over 150 groups that focus on environmental and public health issues, asked the University of North Carolina – Chapel Hill Outreach Core for research support in gathering information on the public health impacts of electronics waste, particularly its hazardous constituents. Outreach Core staff members and students researched this issue, as well as the management practices of other states and compiled a report for ConNet. The organization used this information to identify waste management alternatives with the lowest public health impacts, and then created a fact sheet to educate policy-makers and drafted a proposal for electronics waste management in North Carolina. ConNet's efforts in the introduction of legislation that would ban the disposal of electronics waste in landfills and would implement an advanced recovery fee on electronics sold in North Carolina.

Grady McCallie, environmental liaison for ConNet and one of UNC's Outreach collaborators, sums up the UNC contributions as follows:

> "We rely on the UNC-Chapel Hill SBRP outreach program for trustworthy information about the public health aspects of environmental issues. They translate obscure scientific research into language we can understand, and provide access to experts across the country. Their work makes it possible for us to be credible, fact-based advocates – which results in better protection for our communities and the environment."

In a separate project UNC-CH Outreach staff developed K-12 educational activities – the *What's in the Water?* curriculum. This resource was shared with teachers in Wake and Edgecombe Counties, reaching over 1300 students. One of UNC's educational highlights was a collaboration with the Carolina Center for Public Service to sponsor the workshop in Edgecombe County, which is a predominantly minority county with almost 20 percent of its families having incomes below the poverty level. With CCPS support, the UNC Outreach Core provided instructional modules valued at $200 for each middle school in Edgecombe County.

[**Gray** (**University of North Carolina – Chapel Hill**)]

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4j. Success Stories: SBRP-Funded Graduate and Post-Doctoral Fellows

SBRP is committed to more than the nurturing of an active multidisciplinary, university-based research and outreach program. We also support the long-term advancement of the Program's mission by sponsoring interdisciplinary training and development of emerging scientists in the field of environmental health sciences.

We believe that training is an area of importance to the overall performance of the Program, and to the future of environmental health research in general. The Program provides graduate students and post-doctoral fellows with advanced training in the biomedical sciences, epidemiology, ecology, and geosciences (including hydrogeology, geologic engineering, geophysics, geochemistry, and related fields). More importantly, grantees are encouraged to build programs that result in structured, interdisciplinary training as part of their overall program. It is the SBRP's intent to train students and post-doctoral fellows in the non-biomedical projects in the context of environmental health sciences and biomedical research, as well as giving students of the biomedical sciences opportunities to receive training in the non-biomedical areas of study.

This interdisciplinary approach is important as scientific developments are increasingly dependent on cross-fertilization, which enables researchers to work at the edge of their field and to be exposed to different perspectives and approaches for conducting research. We believe that the SBRP's unique interdisciplinary, multidisciplinary approach is valuable in broadening trainees' knowledge of environmental health sciences beyond what is required by specific degree programs.

> *"Perhaps the most important group of individuals to benefit are the graduate students and postdoctoral fellows who have conducted research on SBRP projects. They have seen first-hand that collaborative research yields value-added results to individual research efforts. I expect that these young scientists are unlikely to ever shy away from an opportunity to expand their research by collaborating with other scientists. The Core laboratory in our grant that is testing the biological activity of products produced in current and experimental site remediation processes exhibits, most strikingly, the melding of disciplines that collaborative research can bring. Environmental engineering students are performing biological experiments in the laboratories of biomedical scientists to examine whether the products produced in experimental remediation systems show evidence of a loss of toxic activity. The major benefits of this type of cross-disciplinary activity are not easily measured in the short run but will be observed in the productive careers of these young scientists as they leave to enter the increasingly complex world that characterizes their future."*
>
> — Larry Fischer, Michigan State University

SBRP-funded researchers have told us that they believe that the Program's unique multidisciplinary training is instrumental in preparing graduates for the leadership positions that SBRP trainees have assumed early in their careers. The following vignettes support this sentiment.

Tom Webster, former post-doctoral associate at Boston University, current SBRP Project Leader, writes about his SBRP-funded training:

"The interdisciplinary nature of the Superfund Basic Research Program was critical to my training and the development of my career. My research relies on applying novel mathematical and statistical tools to individual-level epidemiological data with extensive residential histories, a very important resource. The collaboration between various projects of Boston University's SBRP Center was essential."

David Ozonoff, Program Director of the Boston University SBRP grant program, writes about Chris Paulu (Epidemiologist with the State of Maine Bureau of Health):

"Dr. Paulu's experience with the multidisciplinary approach of the SBRP made him particularly well suited to his present role of epidemiologist within a toxicology group. His exposure to toxicology and molecular epidemiology in the SBRP, combined with his research concentration in environmental epidemiology, has given him the capability to work as an applied toxicologist - a public health function that is much needed at the state level."

Richard Monson, Program Director of the Harvard University SBRP grant program, writes about Robert Wright (Faculty of Harvard Medical School in the Department of Pediatrics):

"The multidisciplinary training he has received through his SBRP training included acquiring expertise in infant developmental and cognitive assessment, exposure assessment, community interaction, and statistical analysis. Together with his training in genetics, this will give him the skills necessary to conduct complex studies of gene-environment interaction in child development."

Dr. Harvey Checkoway, Program Director of the University of Washington SBRP grant program, writes about Blakely Adair (Post-Doctoral researcher at the USEPA's National Health & Environmental Effects Research Lab, RTP, NC):

"Dr. Adair benefited from the SBRP's multidisciplinary training, as can be seen in the well integrated approaches of her studies. The combination of analytical, biochemical and GIS techniques that she learned through the program, combined with her training in the remediation and regulatory arenas, led to her completion of complex research projects with substantial real world relevance to the Superfund process."

Lawrence Fischer, Program Director of the Michigan State University SBRP grant program, writes about Jon MacDonagh-Dumler (Project Manager for Regional Coordination for the Great Lakes Commission):

"After graduation, Dr. MacDonagh-Dumler was hired by the Great Lakes Commission as Project Manager for Regional Coordination, a position he still holds. In this position, he has utilized the training and experience he gained at MSU to carry out his responsibilities with respect to Great Lakes environmental issues. These include: providing regional leadership, generating consensus on regional priorities among diverse interests, and building partnerships for decisive regional action. In this role, he has communicated with key congressional leadership, introduced congressional staff to major Great Lakes issues, and helped lay the groundwork for a consensus-based Great Lakes Restoration Plan. As should be clear from these responsibilities and activities, they require a solid technical background as well as knowledge of the political and economic implications of environmental water policies - a mix of skills that was nurtured through the SBRP multidisciplinary training Jon received."

Martyn Smith, Program Director of the University of California – Berkeley SBRP grant program, writes about Steve Buckley (Assistant Professor, University of Maryland):

"While at Berkeley, Steve took advantage of the opportunities afforded him with his Superfund support to study environmental health science and policy, environmental engineering and air pollution science. Because of his exposure to toxicology, epidemiology and other health sciences, Steve developed an interest in, and facility for, addressing both science and policy issues related to air toxics, energy and combustion."

Dr. Harvey Checkoway, Program Director of the University of Washington SBRP grant program, writes about Therese Mar (Post-Doctoral Research Associate, Department of Environmental Health at the University of Washington):

"The unique multidisciplinary training opportunities offered under the SBRP have been crucial in Dr. Mar's professional development. She entered the graduate program in the department with an undergraduate degree in physics and a Master of Science degree in applied mathematics. During the course of her doctoral studies she took basic and advanced courses in physiology, toxicology and analytical chemistry that provided skills essential to her research project. Moreover, her role in the Superfund project resulted in her working directly with faculty specialists in toxicology, physiology, chemistry and applied mathematics. As a result, Dr. Mar now has very solid education and experience in the application of each of these disciplines. She currently is working as a post-doctoral research associate in the laboratory of Dr. Jane Koenig at the University of Washington. Her strong multidisciplinary training under the SBRP makes Dr. Mar especially well qualified for this important area of public health research."

Kathleen Dixon, Program Director of the University of Cincinnati SBRP grant program, writes about Tat Ebihara (Assistant Professor in the Department of Civil and Environmental Engineering at the University of Kansas):

> *"Dr. Ebihara was an exemplary trainee in our program, who benefited greatly from interactions with faculty and other trainees outside of his immediate research area, including exposure to molecular biology, microbiology, analytical chemistry, and toxicology . . . He is currently supervising an active research program, with funding from both the USEPA and NSF, that is focusing on several research areas that overlap with both environmental health and public health."*

The following descriptions illustrate the types of positions that SBRP-supported students have pursued and highlight some of the contributions SBRP trainees are making in the environmental health sciences. This is only a representative sample of recent graduates; we are currently building a database for tracking student placement and advancement.

The following graduates of SBRP-funded research programs are employed by state or regional environmental agencies:

- Chris Paulu, D.Sc. (Boston University) is working for the State of Maine Bureau of Health. As the primary epidemiologist within the Environmental Toxicology Program, he is responsible for guiding the Program's research studies and surveillance projects.
- Kurunthachalam Kannan (Michigan State University) is a Research Scientist at New York State Department of Health where he manages a laboratory and advises students for their doctoral degrees.
- Jon MacDonagh-Dumler (Michigan State University) is a Project Manager for Regional Coordination for the Great Lakes Commission, providing regional leadership, generating consensus on regional priorities among diverse interests, and building partnerships for decisive regional action.

The following graduates of SBRP-funded research programs are employed by private firms:

- Tyson D. Carlson (University of Arizona) is a Licensed Professional Hydrogeologist in the state of Washington working for Hart Crowser, Inc., a private geotechnical and environmental consulting firm in Seattle working on projects involving contaminant remediation, water quality monitoring, water supply, and construction dewatering design.
- Grantley D. Charles (University of Florida) works in the Toxicology and Environmental Research Department of Dow Chemical Company, Midland, MI where he continues to work in the area of endocrine disruption, developing methods for assessing the estrogenicity and other toxicities of chemicals.

The following graduates of SBRP-funded research programs have university faculty positions in the United States or abroad:

- Thomas F. Webster, D.Sc. (Boston University) is on the faculty of Boston University School of Public Health, Department of Environmental Health. Dr. Webster is a Research Associate on the SBRP-funded project "Detecting and Analyzing Patterns in Spatial Data".
- Dr. Timberley Roane (University of Arizona) is an Assistant Professor in the Department of Biology at the University of Colorado-Denver. She has been awarded the College of Liberal Arts and Sciences Teaching Award and was recently highlighted as Researcher of the Month.
- Dr. Robert Wright (Harvard University) is a pediatrician and medical toxicologist. Dr. Wright was a SBRP fellow from 1998 to 2000. He first served on the faculty of Brown Medical School in the department of Pediatrics and in 2002 he joined the faculty of the Harvard Medical School in the department of Pediatrics, with a secondary appointment at the Harvard School of Public Health in Environmental Epidemiology. He recently became project leader of the Community Based Prevention and Intervention Project of the Harvard SBRP program project grant and is active in community based research efforts at HSPH.
- Steve Buckley (University of California – Berkeley) after completing a post-doctoral research position, served as a Staff Scientist at Sandia National Laboratory and is now an Assistant Professor at the University of Maryland. He is serving as the Environment Division Chair of the American Society of Mechanical Engineering as of July 2002, and also serves as the air pollution chair and the ASME liaison to the AWMA.
- Paul Imhoff (University of North Carolina – Chapel Hill) is a recently tenured Assistant Professor in the Civil and Environmental Engineering Department at the University of Delaware. He has made significant contributions to the study of mass transfer and contaminant transport in subsurface systems. The quality of his research was recognized through a CAREER award from the National Science Foundation.
- Tat Ebihara (University of Cincinnati) is an Assistant Professor in the Department of Civil and Environmental Engineering at the University of Kansas, with teaching, research, and service responsibilities. He is currently supervising an active research program, with funding from both the USEPA and NSF, that is focusing on several research areas that overlap with both environmental health and public health.
- Jun Nakamura (University of North Carolina – Chapel Hill) is currently a Research Assistant Professor at UNC. He has developed several very sensitive new assays for oxidative DNA damage that are being utilized to understand how some of the Superfund-relevant hazardous chemicals cause cancer. This work has accelerated and advanced the science in pursuit of the objectives of SBRP-funded research at UNC and by other researchers beyond UNC.

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4k. Contributions of the SBRP's Multidisciplinary Research Approach

The scientific challenges we face in environmental health sciences transcend the boundaries of any one discipline. By definition, a Superfund site presents a very complex problem. Environmental media at these sites may include air, soil, sediment and surface and groundwater that are contaminated by hazardous chemicals, which are invariably mixtures of metals, solvents, and other organic chemicals such as PCBs or PAHs. The research required to provide a foundation for the development of public health and environmental policies to limit or prevent human exposure to these chemical mixtures requires a broad, multidisciplinary approach that goes beyond the traditional boundaries between academic disciplines. In establishing the SBRP, NIEHS chose to create a network of multidisciplinary, interdisciplinary teams of researchers to provide a broad perspective on environmental health research. The goal of establishing multidisciplinary programs is to provide a more comprehensive understanding of the complex environmental issues in order to support state, local, and federal agencies, as well as private organizations and industry in making decisions related to the management of hazardous substances. *Assembling researchers from diverse disciplines to focus on a unifying theme provides the opportunity to advance the science in a more effective manner than could be accomplished by single unrelated projects.*

Synergisms

The requirement for multidisciplinary programs, which include both biomedical and non-biomedical projects, has been a hallmark of the SBRP since its inception. While this approach was not initially favored by some researchers, who expressed concerns about cultural and methodological barriers between disciplines, it has become recognized as a unique feature of the SBRP that has resulted in synergistic interactions to expand the scope of research accomplishments beyond that of a single investigator. The synergistic interactions increase productivity and innovation, resulting in more rapid scientific progress than would otherwise have been possible.

> *"In our wildest dreams, this diverse group of scientists could not have imagined the synergies that have taken place."*
> — Joe Graziano, Columbia University

The SBRP's five-year grant cycle allows for the establishment of long-term collaborations. Scientists from diverse disciplines have the opportunity to work on the same problem for an extended period – and while initially they may view things from widely varying perspectives, as work proceeds and language and concepts are traded, new, novel and often unexpectedly fruitful directions for solving problems may evolve.

SBRP-funded researchers face extremely complex scientific challenges. They have found that experts from different but complementary disciplines have much to teach and to learn from each other as they cross disciplinary boundaries. Collaboration among a diverse community of peers brings to light questions, factors, and perspectives that might not be considered by researchers focused on a single discipline. Interaction among scientists from different disciplines creates opportunities, and challenges scientists to think differently about the way they conduct studies and communicate results. Interactions of chemists, toxicologists and engineers provide a unique and critical perspective to approach the complex issues associated with risk assessment and remediation at Superfund sites.

> *"It is clear to us that the very process of creating and working in multidisciplinary teams forces the participants to engage in the development of more effective science communication skills, better collaborative skills, and a re-examination of the basic assumptions and paradigms of our respective disciplines as we share our knowledge and results with each other and seek to find common ground. This has greatly improved the science of the individual projects of the program, and has focused them or even changed their overall direction and specific aims as a result of these interactions."*
> —Josh Hamilton, Dartmouth College

Impact on Academic Infrastructure

The SBRP has enabled scientists, who previously were only able to envision the value of research and training that permitted faculty and students to work at the interface of the biomedical, ecological, and engineering sciences, to bring their vision to fruition in the university structure. SBRP-funded research programs were often the first truly interdisciplinary research programs at a university and these programs have served as examples to university administrators and to state agency officials of the benefits that can be attained from multidisciplinary research. The value of the multidisciplinary training offered by these programs has been recognized on these campuses and the philosophy has spread – resulting in course materials being revised and updated, cross-over of students into life sciences and engineering classes, and the increased placement of multidisciplinary faculty on important committees.

Since the establishment of SBRP-funded multidisciplinary research programs at Dartmouth College and the University of California-Berkeley, these universities have launched additional multidisciplinary programs:

- The SBRP program at the University of California-Berkeley has led to the establishment of a group of researchers who know one another very well – and this has been instrumental in helping the campus form a new "Institute for the Environment" and the Berkeley Water Institute.

- At Dartmouth, a new Center for Environmental Health grew out of and is based on the model of the successful SBRP program on toxic metals. Dartmouth has committed $1 million in startup funding for this new Center, the central theme of which is to develop interdisciplinary research, education and outreach programs in environmental health sciences. This new Center has grown in its first two years to over 30 investigators with over $18 million per year in direct cost federal grant support for their collaborative research, and has become a model for other interdisciplinary centers at Dartmouth.

The Next Generation of Environmental Scientists

Very few established investigators are trained in multiple disciplines – SBRP-supported trainees truly are and they will form the next generation of researchers. SBRP-funded students and junior researchers benefit greatly from the Program's interdisciplinary activities, and they learn early in their careers the advantages and challenges of the multidisciplinary approach for solving complex problems in environmental health. The central involvement of graduate students in components of a multidisciplinary program is leading to a new breed of scientists equipped to apply a wide range of investigative tools to help solve the complex questions associated with the health threats posed by soil and groundwater contamination.

Many SBRP Program Directors consider the training of future scientists to be one of the most important legacies of the Program. As so eloquently stated by Larry Fischer, in the previous section and synopsized here,

> *"Perhaps the most important group of individuals to benefit are the graduate students and postdoctoral fellow who have conducted research on SBRP project…. I expect that these young scientists are unlikely to ever shy away from an opportunity to expand their research by collaborating with other scientists…The major benefits of this type of cross-disciplinary activity are not easily measured in the short run but will be observed in the productive careers of these young scientists as they leave to enter the increasingly complex world that characterizes their future."*
>
> —Larry Fischer, Michigan State University

Interestingly, SBRP-funded researchers have reported to us that they believe that the multidisciplinary Superfund programs, as depicted on SBRP pages on university websites, have attracted the attention of potential students and postdoctoral fellows from many different fields, many of whom applied to the universities.

Increased Capacity to Serve the Community

The combined insights of scientists from various disciplines working in SBRP-funded programs have enabled these teams of researchers to provide extraordinarily effective advice to state agencies, federal regulators and the public. For example, scientists at Mount Sinai School of Medicine were able to apply their cross-disciplinary collaborations to determine which pollutants will be most important for the future of the Hudson River and the surrounding communities. In a different capacity, the Program has advanced the infrastructure of children's environmental health by bringing together health care professionals, educators and researchers to establish a national agenda for improving the health of children. However, it is important to appreciate that community involvement has reinforced the necessity for multidisciplinary science. As investigators experience "real world" settings, they frequently find that they must expand their view of how to approach science and that teamwork makes a difference…*It would be difficult to single out any of our projects as the one or two most notable overall advances of this program for its eight years of funding. Rather, it is their collective genesis as a result of our multidisciplinary approach, and their combined impact on public health from this nationally recognized program rather than from diverse individuals, which we feel is our greatest overall achievement. It is a clear validation of stated goal of such program projects to achieve a state where "the whole is greater than the sum of its parts."* — Josh Hamilton, Dartmouth College