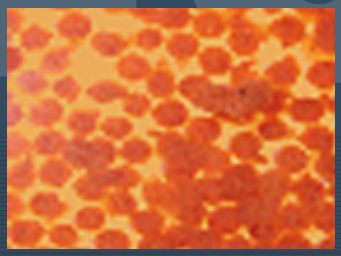
Superfund Basic Research Program

Highlights 2007



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PCBs and Female Reproductive Health: An Enigmatic Paradigm

Brown University SBRP

Project Leader: Surendra Sharma, Ph.D., M.D.

There is growing concern that many man-made compounds, including pesticides, industrial chemicals, plastics, pharmaceuticals, and cosmetics are affecting human health and wildlife populations. Although no longer used, polychloride biphenyls (PCBs) remain ubiquitous environmental contaminants at numerous Superfund sites, posing a serious threat as they either mimic or block the actions of hormones. Based on their endocrine modulating activities, it has been thought that PCBs are potential risk factors for *in utero* effects on perinatal and postnatal health. There are several epidemiological reports suggesting that exposure to PCBs can cause menstrual cycle aberrations, low birth weight deliveries, and postnatal neurocognitive anomalies; however, these effects have not been experimentally confirmed, and there is only a limited mechanistic understanding of this wide spectrum of PCB effects.

Because angiogenesis, trophoblast invasion, fluid regulation, and immune tolerance are the key hormonally-regulated components of normal pregnancy, a team of researchers led by Dr. Surendra Sharma hypothesized that *in utero* exposure to PCBs resulted in inflammatory events leading to perinatal or postnatal effects involving these pathways. They further hypothesized that PCB effects were primarily manifested when coupled with pregnancy-incompatible genetic stress. Dr. Sharma's team studied *in vivo* PCB effects in pregnant wild type or IL-10 knockout (IL-10 KO) mice. Aroclor 1254, when injected interperitoned from gestational days (gd) 4 through 12 at a dose of 500 µg/mouse, did not affect the pregnancy outcome in wild type mice, whereas IL-10 KO mice experienced pre-term birth on gd 17, a two-fold increase in amniotic fluid, decreased placental weight, and reduced litter size with low birth weight and neurocognitive defects. Based on gas chromatographic analysis, all Aroclor 1254 congeners were detected in the utero-placental tissue. It was found that Aroclor 1254 exposure reduced the expression of Aquaporin 1 protein in the placenta. Aroclor 1254 exposure also inhibited VEGF-mediated signaling as demonstrated by reduced VEGF R2 expression and phosphorylation, as well as by disruption of capillary tube formation in a three dimensional culture system on matrigel. Aquaporin 1 and VEGF functions are critical to angiogenesis; their modulation at the maternal-fetal interface is a potential pathway for altered amniotic fluid volume and adverse pregnancy outcome in IL-10 KO mice.

For the first time, this work provides evidence that genetic stress and PCB exposure combine to induce dysregulation of Aquaporin 1 and angiogenesis at the maternal-fetal interface with adverse consequences for the developing fetus.

Arsenic as an Endocrine Disruptor

Dartmouth College SBRP

Project Leader: Joshua Hamilton, Ph.D.

A team of Dartmouth Medical School (DMS) researchers determined that low doses of arsenic disrupt the activity of a hormone critical in development. This finding provides further evidence that arsenic at low doses (i.e., levels found in U.S. drinking water in some areas) can be harmful.

“Arsenic is a natural, yet pervasive, chemical in the environment; we can’t seem to escape it,” says Joshua Hamilton, one of the authors on this study and the director of the Center for Environmental Health Sciences at Dartmouth and Dartmouth’s Superfund Basic Research Program on Toxic Metals. “By learning how it adversely affects biological processes and at what levels we should be concerned, we will hopefully someday be able to mitigate its impact on human health.” Hamilton is also a professor of pharmacology and toxicology at DMS.

Hamilton and his team, in previous work, learned that arsenic at low doses appears to suppress the ability of all critical steroid receptors, including those for estrogen and testosterone, to respond to their normal hormone signals. Chemicals that disrupt steroid hormone receptor signaling are called endocrine disruptors. Arsenic can disrupt these hormone pathways at extremely low doses equivalent to what many people in the U.S. have in their drinking water.

This study set out to see whether arsenic can also disrupt the activity of two hormone receptors involved in normal development - the retinoic acid receptor and the thyroid hormone receptor, two important members of the larger nuclear hormone receptor family. While the researchers studied the impact on frog development, these two hormone receptors are also vital to human development.

“I believe this is the first demonstration in an animal model that arsenic actually disrupts a developmental process that is regulated by hormones, and it does this at extremely low doses that are directly relevant to human exposures of concern,” says Hamilton.

Developmental Neurotoxicity of Superfund Pesticide: Biomarkers and Mechanisms

Duke University SBRP

Project Leader: Theodore Slotkin, Ph.D.

Exposure of pregnant women and children to pesticides and other Superfund chemicals represents one of the major environmental concerns for effects on neurodevelopment. There is a need to develop rapid screening techniques to enable us to focus on chemicals that are likely to be of the greatest potential danger. In this project, the Duke University SBRP is comparing defined developmental neurotoxicants, such as the organophosphate pesticides, with other suspected classes of neurotoxicants, such as carbamate and organochlorine pesticides, heavy metal contaminants, and river sediments from contaminated sites. In 2007, the lab designed an *in vitro* test

system using PC12 cells, a cell line that recapitulates the critical stages of neuronal development ranging from cell replication through differentiation and axonogenesis, and the specification of particular neurotransmitter phenotypes known from earlier work on this project, which will be targeted by chlorpyrifos and related organophosphates *in vivo*.

Dr. Slotkin's group completed comparative studies of different organophosphates that target neural systems in developing rats, including the identification of critical periods of vulnerability of specific brain regions and the characterization of the critical mechanisms underlying the effects. With the *in vitro* model, the lab established that other organophosphates are equally damaging to neural cell replication and differentiation at exposure levels below the threshold for significant inhibition of cholinesterase; the effects intensify as the transition to cell differentiation is approached. Notably, even unrelated toxicants that act through different originating mechanisms can elicit similar patterns of disrupted neural development, including dieldrin (an organochlorine pesticide) and divalent nickel (metal contaminant). The organophosphates, as well as other agents, interfered with the specification of the cholinergic phenotype; these are the type of neurons that are the most sensitive to damage when assessments are conducted *in vivo*. Accordingly, Dr. Slotkin's team identified the critical stages (replication through early differentiation) that are vulnerable to the developmental neurotoxicity of these agents, including the identification of why certain circuits are targeted. With that information, the lab explored methods of amelioration, depicting that a "cocktail" of protectant drugs can prevent developmental neurotoxicity as a result of organophosphate exposure. Finally, scientists continue to explore the events in gene transcription that precede the emergence of neurotoxic outcomes as a way of determining if there is a "fingerprint" pattern of gene expression that predicts whether given agents will be developmental neurotoxicants, and what their likely targets and critical periods might be. The lab has successfully definined gene groupings that make these predictions across different classes of compounds with disparate neural targets.

These results are important for three reasons. First, the adverse effects of chlorpyrifos on neurodevelopment are shared by other organophosphates, and also by apparently unrelated Superfund chemicals that can target similar events, such as divalent heavy metals and organochlorines. Second, in the case of the organophosphates, these studies point to mechanisms, other than cholinesterase inhibition, as the primary culprits, so the current use of cholinesterase activity as a biomarker for these adverse effects is inappropriate, necessitating a revised approach to the detection of developmental neurotoxicity. ***Third, the fact that the developmental neurotoxicity of various compounds can be modeled effectively in cell cultures opens the door to rapid screening of suspected environmental neurotoxicants, using a combination of gene transcription "fingerprints" and defined, adverse outcomes.***

The information provided by this project has been cited heavily in important documents for pesticide regulation, including the 2006 EPA Inspector General's Report (Report no. 2006-P-00009), "Opportunities to Improve Data Quality and Children's Health Through the Food Quality

Protection Act." This project served, and continues to serve, as an endpoint guide for studies of neurodevelopment outcomes in children exposed to pesticides, cited by a number of NIEHS-funded research centers.

Arsenic Biomarker Epidemiology

University of California, Berkeley

Project Leaders: Allan Smith, M.D., Ph.D., and Martyn Smith, Ph.D.

This project explores the effects of childhood arsenic exposure and the mechanisms that may confer susceptibility to these effects. The Arsenic Health Effects Research Program, led by Dr. Allan Smith, has shown that *in utero* and early childhood exposure to arsenic results in major increases in lung cancer and bronchiectasis mortality in young adults aged 30-49. The program also reported a three-fold increase in mortality from acute myocardial infarction in young adults who had early life exposure to arsenic in drinking water. These findings indicate that early life environmental exposure to arsenic in drinking water is strongly linked to disease incidence and mortality in adulthood.

Assessing the Adverse Effects of Environmental Hazards on the Reproductive Health in Human Populations

University of California, Davis

Project Leader: Bill L. Lasley, Ph.D.

The term "endocrine disrupting substance" (EDS) was coined in 1991 in an effort to categorize a specific type of environmental toxicant. These compounds exert their action by interfering with normal hormone action and perturb the functions of tissues and organs particularly those of the reproductive tract. Given the variety of their structures and distinct biological effects, it is not surprising that a variety of potential mechanisms are proposed to explain how EDSs influence the endocrine system. Most of the compounds that have been identified to date interfere with estrogens and are referred to as "environmental estrogens". In general, these compounds act as hormone "mimics" and have only weak hormone activity on their own but tend to block the action of natural hormones in animal models. The classic examples of EDS/environmental estrogens are the metabolites of DDT which, in birds, cause weakening of egg shells through blockage of estrogen action in the shell gland. While androgenic hormones are structurally similar to estrogenic hormones, significantly fewer compounds have been associated with androgen action despite the repeated observations by epidemiologists that several widespread human health issues seem to be related to a form of yet to be explained androgen disruption. Examples of these unexplained issues are the decline in human sperm production, an increase in prostatic diseases, breast development at a younger age, and virilization of peri-pubertal girls. These abnormalities are logically related to an increase in hormone action rather than a suppression of hormone action which has been the prevailing dogma associated with previously defined EDSs and this inconsistency has prevented known EDSs from being associated with this set of health problems. Advances in our understanding of the underlying mechanisms of a variety of EDSs have resulted in the development of new tools and models for studying a wide range of molecular structures.

A significant breakthrough by ***Dr. Lasley's group at the UC-Davis Superfund Basic Research Program recently identified triclocarban (TCC) and closely related compounds as a new type of EDS that have endocrine qualities consistent with the unexplained human health issues listed above.*** The unique quality that allows these compounds to be categorized as a "new type" of EDS is that instead of acting as "mimics" and blocking hormone action, these compounds act to "augment" or "enhance" the normal action of the natural hormone. Somewhat surprisingly, some of these compounds are frequent additives in personal care products such as soaps, shampoos and toothpaste and are, therefore, in frequent contact with human skin, hair and mouth, resulting in common human exposures. Scientists previously failed to recognize the potential hazard presented by TCC and related compounds because of their inability to simulate and investigate the unique endocrine disruptive properties of these compounds with a reliable laboratory test. The development of a novel cell-based bioassay for androgens and the insight to explore a previously unsuspected "augmentational" effect of small molecules on steroid action were the key elements that led to this discovery. Subsequent *in vivo* studies using rats demonstrated that the enhancing effects of TCC seen *in vitro* with cells are also induced when TCC is added to the diet of male rats. In these animal studies, there was essentially no effect when TCC was administered alone; however, all androgen-sensitive target organs were larger when both TCC and testosterone were administered when compared to testosterone alone.

This discovery underscores the complexity of EDS mediated nuclear receptor transcription and highlights the need to reassess the criteria for classifying a compound as an EDS. The growing number of potential modes of EDS action that do not directly affect ligand binding or receptor activation raises the concern that the EDS property of these compounds is unlikely to be predicted by conventional quantitative structure-activity relationship modeling or simple receptor activation assays. The recognition of these compounds as environmental hazards that can possibly enhance a wide range of hormone actions will provide new directions for future investigations which attempt to explain lowered sperm production, increased prostate disease and metabolic changes in younger women.

Thousands of new compounds are produced for commercial use each year. Many are screened for potential toxic effects, but many are not properly examined for lack of practical screening technologies. The fact that this study identifies a commonly used and widely applied compound as potentially harmful underscores the importance of this new technology that was developed and applied in a particular way. Clearly, this technology or a reasonable facsimile will be employed in the future as a part of chemical screening for reproductive and/or metabolic hazard potential both in chemical production and hazard disposal.

Better Remediation Through Chemistry: Increasing the Efficacy of Pump and Treat Remediation Strategies for Arsenic Contaminated Sites

Columbia University SBRP
Project Leader: Steven Chillrud, Ph.D.

Arsenic is a prevalent contaminant at U.S. Superfund sites, present at more than 500 sites. "Pump and treat" remediation is commonly used at contaminated groundwater sites, with more than 900 such sites nationwide. However, arsenic remediation by pump and treat systems is often complicated by the relatively slow release of arsenic from iron and aluminum (hydr)oxides on the surface of aquifer solids. As such, sites utilizing pump and treat require many decades to reach desired clean-up levels, and continue to have high annual costs.

The Vineland Chemical Company, a biocide manufacturing facility, located in southern NJ, improperly stored and disposed of their arsenic chemicals for several decades. This led to extensive arsenic contamination of subsurface soils and groundwater, in addition to offsite arsenic transport. At the Vineland Superfund Site, a large pump and treat remediation facility (~ 2 million gallons/day) began operation in 2000, and has significantly reduced offsite arsenic transport. It also led to an initial decrease in groundwater total arsenic concentrations in the pumping wells by as much as 10 times. Several more years of pump and treat, as well as remediation efforts focused on excavation, cleaning, and replacing contaminated vadose zone soils, have led to only relatively minor additional improvements in groundwater arsenic levels, which can have more than 1000 ppb As. The aquifer sediments are still elevated in arsenic and represent a reservoir of approximately 100,000 kilograms of arsenic at the Vineland site, causing continued groundwater contamination.

Columbia University scientists hypothesized that the timeframe for remediation of arsenic contaminated aquifers via pump and treat could be substantially reduced by subsurface additions of chemical amendments. The goal is to increase the mobilization of arsenic from aquifer solids and increase the amount of arsenic being removed from the aquifer with each volume of water pumped out of the ground and treated. To date, the scientists have primarily focused on laboratory experiments designed to evaluate the efficacy of chemical amendments for mobilizing arsenic from aquifer solids. The most promising chemical amendments are sodium phosphate and oxalic acid. Phosphate has a similar chemical structure to arsenate, and can replace arsenic sorbed into the surfaces of aquifer minerals. Oxalic acid is a natural soil acid that is responsible for dissolving and transporting iron and aluminum oxides in vadose zone soils (these phases are also important on aquifer mineral surfaces).

Column experiments using arsenic-contaminated aquifer sediment (~80 ppm arsenic) from Vineland suggest that both phosphate and oxalic acid can be significantly effective at increasing arsenic mobility compared to groundwater alone. The effluent water arsenic concentrations in these column

experiments reached 100,000 ppb and 70,000 ppb for 10 mM oxalic acid and 100 mM phosphate experiments, respectively, whereas the influent arsenic concentration was only ~10 ppb. ***More importantly, extrapolations for site clean-up progress, using data from these column experiments, suggest that chemical treatments could lower the site's clean-up timescale from ~600 years with ambient groundwater alone to only four years with 10 mM oxalic acid, based on the clean-up goal of lowering the concentration of arsenic on aquifer solids to less than 20 ppm.***

The Columbia scientists are now planning *in situ* field experiments using subsurface chemical amendments to build on these laboratory results. Modeling groundwater flow and transport at the site has helped design optimal locations for injection and monitoring wells within the cone of depression of a site recovery well. Furthermore, it has helped constrain amendment concentrations, injection rates, and pumping rates needed for these experiments. Before carrying out the injection of chemical amendments, the investigators will first perform inert tracer experiments to confirm the conclusions from numerical modeling efforts, and to build confidence that any chemicals added will be recovered at the existing large-volume recovery well.

The Columbia scientists also plan to investigate several basic science questions directed towards both the mechanisms by which the oxalic acid mobilizes arsenic, as well as the eventual rates of microbial degradation and sorption of oxalic acid.

Chemical amendments appear to show great promise in dramatically reducing the time required to use pump and treat intervention to remediate the Vineland megasite and, potentially, many other sites with arsenic contaminated aquifers. This would be an important innovation since pump and treat options can be quite an expensive technique. A 2003 survey of annual operation and maintenance costs using pump and treat reported a mean annual cost of ~$0.6M, with the Vineland Superfund pump and treatment costs being the highest surveyed at $4M/yr.

Nanotechnology-based Environmental Sensing

University of California, Berkeley

Project Leaders: Catherine Koshland, Ph.D., and Donald Lucas, Ph.D.

This project (which is the doctoral thesis work of student Marvin Mulvihill) made significant progress towards developing a functional arsenic detector in groundwater using nanotechnology. Using close-pack arrays of octahedral shaped silver nanocrystals, the chemical sensing of arsenic ions, with detection limits as low as one part per billion in solution, was possible. The sensor is based on surface-enhanced Raman spectroscopy (SERS), where analyte molecules near nanostructured metallic surfaces experience huge enhancements in Raman scattering cross-sections, typically orders of magnitude higher than expected. The nature of vibrational spectroscopy allows for the simultaneous measurement of arsenic in its common oxidation states, and can discriminate between the two. This is important since different oxidation states have different toxicities.

Detection is performed directly on the substrate by placing a droplet of the analyte solution onto the nanocrystal monolayer. No additional sample preparation is required. These substrates have been verified to work in the presence of known competing anions, and accurately characterize arsenic levels in polluted well-water obtained by collaborators in Nevada.

Transport, Transformation and Remediation of Contaminants in the Environment: Exposure Assessment in Heterogeneous Environmental Media

University of California, Davis
Project Leader: Kate M. Scow, Ph.D.

Between the mid-1980s and 1997, citizens of the City of Rancho Cordova (California) were exposed to previously unknown levels of perchlorate in their drinking water (obtained from groundwater). The human exposure halted in 1997 when the contamination was discovered and numerous wells were shutdown. The groundwater perchlorate contamination occurred, primarily, as a result of the reinjection of treated groundwater produced by ongoing remediation of TCE plumes. For much of the potentially exposed population, the California Department of Health Services had data on neonatal thyroxine (T4) levels at birth from 1985-1997. T4 levels at birth can indicate prenatal exposure to perchlorate; however, to determine a cause-and-effect relationship between exposure and health effects (epidemiology), one must also estimate possible exposure levels for each individual as a function of space (residence location) and time.

This study used new hydrologic modeling tools developed in a previous Superfund research project with the above-mentioned epidemiological data, and in cooperation with the California Department of Health Services, in order to mathematically simulate exposure levels, including uncertainty, and assess whether a statistically significant health effect occurred. In essence, ***advanced hydrologic modeling was used to reconstruct the possible exposure levels at each residence in the affected area of the community so that the epidemiological study could be conducted.*** The following are some unique aspects of this work: (1) it is the first exposure analysis to include effects of uncertainty of the subsurface complexity (geology) on modeled exposure levels at residences; (2) it is one of the first groundwater modeling studies to couple the groundwater model dynamically to a surface distribution model that routes the pumped groundwater to each residence in the affected area of the community; and (3) it is the first study to attempt to use results of a probabilistic groundwater-surface distribution model to estimates human exposure in an epidemiological context.

Consequently, this work has provided important insights into state-of-the-art groundwater modeling, and future research needs at the interface between probabilistic groundwater exposure modeling and epidemiology. Ultimately, after continued refinement of this analysis, the results could lead to a better-defined maximum contaminant levels for perchlorate.

Results of the hydrologic modeling indicate that the greatest source of uncertainty in the exposure estimates is the subsurface heterogeneity: distribution of aquifer (e.g., sands and gravels) and non-aquifer (e.g., silts and clays) materials; and hourly to daily fluctuations in pumping rates of wells. For some wells near the outer margins of the perchlorate plume, this uncertainty includes the finite probability that the wells received no contamination, even though the mean model result for these wells indicated some expected contamination. The best way of incorporating such probabilistic estimates of exposure into epidemiological analyses is still being investigated. The modeling results indicate that uncertainty in the surface distribution model is not as significant as uncertainty in the groundwater model.

Average maternal exposures to perchlorate during the third trimester of gestation were evaluated for their effect on neonatal thyroxine (T4) levels at birth (n= 4,299)using the hydrologic models. Results show no statistically significant correlation between modeled perchlorate exposure and T4 levels. However, results do show a tendency for increased T4 in response to high perchlorate exposure, which conflicts with the literature and needs further investigation. Preliminary findings are that all exposure realizations in the lowest dose (> 0 – <6 ppb) showed slight decreases in T4, and in the higher exposure categories (100 – <200, 200+ ppb) point estimates tended to show an increase in T4. The lack of a statistically significant effect is likely either due to lack of a true effect or masking of the true effect by inaccuracies in estimates of exposure assessment. Future studies will therefore focus on the propagation of hydrologic model uncertainty into the exposure and epidemiological analyses. Work is ongoing to properly evaluate the statistical confidence intervals and whether greater variance of response (T4) at the higher exposure levels is indicative of wider confidence intervals or is to be expected because of greater spatial variance of concentrations in highly exposed of the aquifer and other factors.

Evaluation of Tools for Monitoring Natural Attenuation of MTBE

University of California, Davis
Project Leader: Graham E. Fogg, Ph.D.

In California alone there exist tens of thousands of sites where petroleum compounds have leaked in significant volumes into the subsurface, mostly from leaking underground fuel tanks (LUFT). Past use of MTBE at significant volumes in gasoline has complicated this problem because MTBE is mobile and relatively resistant to biodegradation under most natural groundwater conditions. Because both gasoline tanks and public water supply wells are often collocated in population centers, and because groundwater travels to wells on decadal or greater time scales, water resource managers must concern themselves with current and future impacts of MTBE and an associated compound, tert-Butyl alcohol (TBA), on groundwater quality. Many fuel tank leaks are not detected until private or public drinking water supply wells are impacted. Similar circumstances exist in many other regions of the U.S. as well as in Europe.

Natural attenuation of a groundwater contaminant refers to the dissipation, diminishment and immobilization of contaminant mass due to physical, chemical and biological processes. In some contexts, the dissipation of concentrations through dispersion (dilution) is also considered a natural attenuation mechanism. For all but a small number of groundwater contaminants, natural attenuation alone is insufficient to mitigate the contamination and protect groundwater quality. It is theoretically possible, however, to monitor groundwater contaminant plumes so as to estimate whether significant natural attenuation is occurring, depending strongly on complexity of the subsurface relative to the number of monitoring wells and on whether data can be collected that conclusively indicate whether significant natural attenuation is proceeding. Previous work by SBRP funded researchers at UC-Davis demonstrated that data from even high densities of monitoring wells can easily give the appearance of natural attenuation where none is happening, because of complex, unmodeled plume migration and dispersion processes.

Two potentially useful tools to detect significant natural attenuation even when the monitoring well data are sparse are analysis of the TBA/MTBE ratios and MTBE carbon and hydrogen isotope ratios ($^{13}C/^{12}C$ and D/H). Since one source of TBA in the LUFT plumes is degradation of MTBE, the TBA/MTBE ratios would be expected to rise if MTBE is biodegrading. Furthermore, because biodegradation will tend to favor transformation of the lighter carbon isotopes, the $^{13}C/^{12}C$ ratio will tend to rise if biodegradation is occurring. Such tools would provide unequivocal evidence of in-situ biodegradation of MTBE *if* no other processes could be expected to cause similar shifts in the ratios. Recent research has identified such processes and explained how neglecting them can lead to erroneous conclusions regarding natural attenuation.

Specifically, this research on effects of commonly used site remediation strategies relying on volatilization, e.g., soil vapor extraction (SVE) and dual phase extraction (DPE), on TBA/MTBE ratios shows that SVE causes significant increases in TBA/MTBE ratios that have been interpreted in the literature to indicate MTBE biodegradation at numerous sites. This effect is readily predictable from principles of chemistry (Raoult's Law) and the well known properties of these compounds. Simulations of petroleum hydrocarbon fate in the soil, vapor, NAPL (non-aqueous phase liquid) and aqueous phases using the model VENT3D demonstrate that SVE can cause significant shifts in TBA/MTBE ratios. Therefore, the widespread observations of increases in TBA/MTBE could be due to preferential stripping of MTBE from the shallow subsurface via SVE remediation rather than due to natural biodegradation. Concerning the utility of $^{13}C/^{12}C$ ratios for indicating MTBE biodegradation, the researchers developed a suite of models for evaluating under a variety of field conditions whether differential rates of diffusion and transverse mixing of the two isotopes could cause enough change in the ratio (fractionation) to confound the interpretation with respect to biodegradation. Model simulations show that under a wide variety of circumstances, molecular diffusion and dispersion can indeed increase $^{13}C/^{12}C$ ratios, especially toward the leading edge of a contaminant plume. The model results are consistent with observed $^{13}C/^{12}C$ data from aerobic biodegradation in laboratory microcosms. Consequently, in some cases the interpretation of field $^{13}C/^{12}C$ ratios for identifying

MTBE biodegradation can be problematic without careful analysis of the accompanying physical transport processes.

Successful Treatment of an MTBE-contaminated Drinking Water Aquifer Using an *Ex Situ* Bioreactor Naturally Colonized by Native Aquifer Bacteria

University of California, Davis
Project Leader: Kate M. Scow, Ph.D.

The fuel oxygenate, methyl tertiary butyl ether (MTBE), has contaminated numerous groundwater aquifers in the U.S., including a number of drinking water aquifers. Despite the fact that MTBE is no longer used as an additive, residual contamination from previous spills is widespread and persistent at many locations. This contamination results in major economic losses due to closure of municipal wells and the requirement to import alternate sources of water for public consumption. ***A fruitful collaboration between the research program of SBRP Project Investigator Dr. Kate Scow at UC Davis, Tesoro Petroleum, and Haley & Aldrich Engineers brought an emerging technology into practice that led to the successful and cost-effective bioremediation of an MTBE-contaminated major drinking water aquifer in North Hollywood, CA***.

The impacted San Fernando Valley aquifer, 200 ft below ground, was contaminated by thousands of gallons of gasoline released from a service station in North Hollywood. The MTBE plume emanating from the original area of contamination had migrated away from the station towards major municipal water supply wells. MTBE concentrations in groundwater near the source were greater than 10 mg/L (4 orders of magnitude > drinking water quality standards). The plume had migrated below a residential area where there was little space, and likely minimal public support, to construct a full-scale pump and treatment system.

In a pilot study, a small scale, *ex situ* bioreactor filled with granulated activated carbon was constructed to explore the feasibility of using bioremediation to treat contaminated water pumped up from the aquifer. The original plan was to inoculate the reactor with a known MTBE-degrading bacterial inoculum. Unexpectedly, however, the bioreactor was colonized by native microbial communities that apparently enter the reactor with the pumped-in groundwater. Almost 100% removal of MTBE was established after about 1 month and was maintained. Removal rates were so high that the pilot bioreactor was successful in treatment of the MTBE plume in the aquifer without addition of non-indigenous organisms.

Using molecular tools developed in Dr. Scow's lab, the presence of high numbers of an MTBE-degrading bacterial strain, *Methylibium petroleiphilum* PM1, was detected in the bioreactor and lower populations were found to occur naturally in the North Hollywood aquifer. PM1, an MTBE-degrading bacterium discovered in Scow's lab, can degrade MTBE to carbon dioxide, without accumulation of toxic metabolites, and has been found to be naturally present at a number of MTBE-contaminated

sites. In the reactor, *population densities of PM1 were related to MTBE treatment efficiency*. Working closely with the Upper Los Angeles River Area Water master and regulators from the Regional Water Quality Control Board, permission was obtained to re-inject the treated water, that now met drinking water quality targets for MTBE, back into the aquifer (rather than discharge it to a sewage treatment plant which was standard practice), in part because no non-native organisms had been introduced into the reactor. This project was the first system to be approved for re-injection of treated effluent into a drinking water aquifer in California.

This study demonstrates that inoculation of bioreactors with non-native bacteria, often associated with high costs and complex regulatory approval processes, may not be necessary at sites where native MTBE-degraders are present. In addition, the added benefit of re-injecting clean water into the aquifer helps to sustain important and sometimes dwindling water resources. This is significant because the contamination of public water supply wells has serious economic and community impacts and these include replacement costs that are estimated to be $7 million per well. Understanding and harnessing the metabolic potential of native organisms can help lead to implementation of low-cost remediation technologies and streamline future bioremediation approval processes.

The project won the award for "Outstanding Project in Ground Water Remediation Award" in 2005 from the National Groundwater Association (NGWA) (http://www.ngwa.org/awards/gwremed.cfm). An article in the LA Daily News by Kerry Cavanaugh, described Dr. Scow's research as: "'Good bugs' cleaning up water tainted with MTBE. The research was also featured on Sacramento News10 at 11 on ABC: http://www.news10.net/storyfull2.aspx?storyid=21166, and in the Fall 2005 newsletter "Earth Works" published by Haley & Aldrich Engineering: http://haleyaldrich.com/knowledge_sharing/resources/earthworks/mtbe.html.

Catalytic Destruction of Perchloroethylene (PCE) and Trichloroethene (TCE) from Soil Vapor Extracted Gases Park-Euclid State Superfund Site Field Trial Conditions

University of Arizona SBRP

Project Leader: Eric Betterton, Ph.D.

Halogenated hydrocarbons, such as the solvents trichloroethylene (TCE) and perchloroethylene (PCE), are among the most commonly found pollutants in the United States water supply. Small amounts can contaminate large volumes of potable groundwater resources because they are toxic at very low concentrations. People exposed to TCE by drinking contaminated water may exhibit a variety of health problems including skin irritations, cancers, birth defects, miscarriages, coordination, and speech and hearing impairment.

Over 90% of the Federal and State Superfund sites contain chlorinated solvents. Sources of contamination are typically associated with electronic manufacturing plants, military facilities, dry cleaners, and old landfills. In most cases, groundwater pollution is the result of storing or disposing

liquid solvent wastes between the years 1940 and 1970. Often TCE and PCE are found in water with other toxic chemicals, such as dichloroethene and vinyl chloride, which are produced by natural degradation of these two chemicals.

One method for treating contaminated sites is to drill a series of wells and then to pump out the air and the volatile contaminants from the soil, called soil vapor extraction (SVE). Most often the contaminants are captured on activated carbon, which eventually becomes saturated with the pollutant and must be transported offsite as a hazardous waste. The pollutant is not destroyed; it is just transferred from the air to the carbon.

Dr. Eric Betterton and colleagues' catalyst technology is designed to destroy, rather than capture, TCE and PCE in soil vapor extraction gases, thus, eliminating or minimizing the need for activated carbon traps. Destruction is based on metal-catalyzed reactions under redox conditions. The catalysts used are similar to the "off the shelf" catalytic converters used in automobiles. ***The resultant gas stream is essentially contaminant free, with solvents converted to carbon dioxide and hydrochloric acid (HCl).*** The most important innovation of this study is the use of redox conditions in which the contaminated soil vapor gas stream is mixed with a few percent propane (a chemical reductant and fuel). This allows the reactor to achieve complete destruction of chlorinated compounds at relatively low temperatures, while, at the same time, maintaining an environment that prevents carbon residue (Coke) formation and catalyst poisoning.

The laboratory tested two pilot-scale reactors to treat SVE gases containing TCE and PCE that contaminate the vadose zone over the underground aquifer at the nearby Park/Euclid field site. Researchers worked in close cooperation with the current owners of the site, the environmental engineers (currently treating the soil vapor with activated carbon), the Pima County Department of Environmental Quality, Arizona State Department of Environmental Quality, and small business (for SBIR Phase 1 funding to partially support this project). Scientists have also worked closely with the local citizens' advisory board.

At the field site, two alumina supported Pt/Rh (platinum/rhodium) catalysts operate at approximately 500 °C to destroy 25 – 200 ppm PCE, and 10 – 50 ppm TCE. The total flow rate is 100 liters per minute giving a residence time of a few hundredths of a second. Propane (1.0 – 2.0%) is added from a tank. Results show near complete destruction of TCE and PCE, without signs of catalyst deactivation over 180 days of almost continuous operation. This efficiency remained even when contaminant inlet concentrations increased. Destruction of chlorinated contaminants using an automobile catalyst is efficient under these unique redox conditions. Catalyst poisoning is minimal; the end products are environmentally benign, including CO_2 and HCl, which are removed by passage through a water scrubbing tower.

Mass-transfer Dynamics of Chlorinated-solvent Immiscible Liquids in Porous Media

University of Arizona SBRP

Project Leader: Mark Brusseau, Ph.D.

Denser-than-water nonaqueous phase liquids (DNAPLs) have been widely used as dry-cleaning solvents, and as degreasing and cleaning agents for military, industrial, and commercial applications. Spillage and improper disposal have resulted in DNAPL contamination of the subsurface, creating a legacy of hazardous waste sites for which remediation is costly and requires lengthy periods of time.

Because DNAPLs do not mix with water, they exist as a separate phase trapped between an impermeable layer and the water above; these zones of trapped DNAPL are called source zones. The amount or flux of DNAPL emanating from a source zone is a primary determinant of the risk associated with a contaminated site. A major question is how much DNAPL must be removed from the source zone to allow a significant reduction in the DNAPL mass flux, which is a key metric for evaluating the effectiveness of a source-zone remediation effort.

Dr. Brusseau's team examined and published data from field studies to evaluate the relationship between DNAPL mass flux reduction and source-zone DNAPL mass removal. The analyzed studies represent a variety of source-zone architectures, DNAPL compositions, and implemented remediation technologies. A total of 21 remediation projects examined DNAPL flux before and after DNAPL source zone mass removal. Mass removals were greater than 60% in most cases. The mass-flux-reduction/mass-removal relationships exhibited an approximate one-to-one behavior for the majority of the sites.

It should be mentioned that these 21 studies only provided a single snapshot concerning DNAPL behavior; therefore, scientists examined data from two sites that were collected on a time-continuous basis. This analysis showed that reductions in mass flux were significantly different for the two sites (90% vs. ~8%) for similar mass removals (< 40%). Further analysis of the data show that low mass flux reduction occurred in the system for which mass removal was relatively efficient (ideal mass transfer). Conversely, a high mass flux reduction was observed for the site for which mass removal was inefficient (nonideal mass transfer). ***These results indicate that source-zone architecture, and associated mass-transfer processes, have great influence on mass flux reduction, and must be considered in planning remediation and in risk analysis.*** The information produced by these researchers is being disseminated to EPA remedial project managers, environmental consultants, and other interested parties through in-house and web-based seminars co-hosted by the EPA, NIEHS SBRP, and UA SBRP.

The relevance and import of this work spans multiple federal and state programs. The research produced under the auspices of the SBRP project has been instrumental in the development of multiple collaborative efforts. For example, the methods this laboratory discussed have been

applied to a large, federal chlorinated-solvent contaminated Superfund site in Tucson, AZ, involving the U.S. Air Force and Raytheon Corporation. We will conduct a similar study at a related site beginning in early 2008, associated with the chlorinated solvents initiative of the US Department of Defense's Strategic Environmental Research and Development Program. In addition, the methods and information employed for a new collaborative initiative were developed by the US Department of Energy, and focuses on chlorinated solvents in the vadose zone.

Hexavalent Chromium (Cr-6)

Brown University SBRP

Project Leader: Anatoly Zhitkovich, Ph.D.

Hexavalent chromium (Cr-6) is a widespread environmental carcinogen that is present as a major toxic contaminant at numerous Superfund sites. In the workplace, exposure to Cr-6 impacts an estimated half million people in the U.S. Cr-6, by itself, is not reactive, and it requires reduction inside cells to cause DNA damage. This reduction process proceeds through the formation of variable amounts of Cr-5 and Cr-4 intermediates and finally yields stable, and DNA damaging Cr-3. The most rapid cellular reducer of Cr-6 is vitamin C, which uniquely converts Cr-6 into Cr-3 by skipping Cr-5 production. For a long time researchers believed that only slow reduction of Cr-6 leading to the buildup of Cr-5 could cause extensive genetic damage. These mechanistic views led to the prediction that only massive doses of Cr-6 depleting cellular vitamin C could cause cancer. This suggestion was further supported by the weak ability of Cr-6 to induce mutations in standard test systems. However, recent epidemiological and risk assessment studies conducted by EPA have found that workers had almost a 25% lifetime risk of dying of lung cancer under a long established permissible exposure limit (PEL) of 52 $\mu g/m^3$ Cr-6. In 2006, OSHA enacted an even lower PEL (10-times), which still resulted in an estimated 10-45 additional deaths per 1000 workers. Thus, epidemiological findings were clearly inconsistent with the mechanistic data and models, introducing a significant uncertainty regarding the risk assessment process (based exclusively on data in human populations with unavoidable secondary exposures).

A peer-reviewed paper published in *Nucleic Acids Research* in 2007 by Brown University SBRP researchers led by Dr. Anatoly Zhitkovich presented novel mechanistic findings clearly demonstrating that Cr-6 is, in fact, highly mutagenic and capable of causing extensive chromosomal breakage, even at very low doses. These investigators discovered that, in order to reveal its very high genotoxic potential, Cr-6 has to be tested in cells supplemented with normal concentrations of vitamin C. Under standard culture conditions, cells are severely deficient in vitamin C and unable to rapidly convert Cr-6 into Cr-3. However, when cells were cultured in the presence of physiological levels of vitamin C, Cr-6 induced DNA damage and chromosomal breaks with a linear dose-response at environmentally relevant concentrations. ***At low environmental doses, Cr-6 produced 10-20 times more chromosomal breaks in cells in the presence of vitamin C as compared to cells lacking this vitamin.*** These novel findings on the high genotoxicity and mutagenicity of Cr-6 provide strong experimental support for the biological plausibility of the carcinogenic effects of this toxic metal at low and moderate levels of exposure. Paradoxically, high amounts of cellular vitamin C that usually exert protective effects against toxic insult by many reactive chemicals were found to strongly enhance genetic damage by carcinogenic Cr-6.

New Methods for Assessing Chemical Mixtures

Boston University SBRP

Project Leader: Tom Webster, Ph.D.

While laboratory toxicology studies typically examine one compound at a time, we, in the real world, are exposed to complex mixtures. Mixtures can be synergistic (having greater than expected effects) or antagonistic (possessing less than expected effects). Importantly, the effects of toxic compounds can also *add* in significant ways. For example, it is possible for mixtures to cause responses even though each component is present at concentrations that do not individually generate measurable effects. Called *concentration addition* (or dose addition), this phenomenon can occur if compounds act in similar ways, for example by activating the same receptor. One can think of receptors as molecular locks, and the compounds activating them as keys. Fitting the key into the lock can regulate certain biological processes.

Concentration addition was first used to examine the interaction of pharmaceutical drugs, but it has applications in toxicology as well. Concentration addition is commonly used in assessing risks from exposure to dioxin and similar acting compounds. Dioxin-like compounds can be assigned a "toxic equivalency factor" (TEF) based on their relative potency, i.e., their relative ability to bind and activate a certain receptor, the AhR receptor. This approach allows risk assessors to examine the total "dioxin-like" activity of complex mixtures that typically include numerous chemicals. Concentration addition could theoretically be applied to a large range of endocrine disruptors, chemicals that interfere with hormones. For example, concentration addition has been used to examine mixtures of compounds that act like estrogen.

Current methods for assessing concentration addition suffer from a very important limitation: they can only be applied to compounds with the same maximal effect (e.g., as the dose of a compound increases, the response typically levels off at some maximum). However, it is common to find compounds whose response plateaus at less than the maximal possible effect. These compounds are called partial agonists, and can include many of the dioxin-like agents for which the TEF method is typically used.

The Boston University (BU) SBRP laboratory developed a method that addresses this problem, and allows for the assessment of compound mixtures with differing maximal effects. The lab's method can also examine the effects of competitive antagonists, that is, compounds that have no effect themselves, but displace compounds that do, resulting in overall reduction of the effect of the mixture. Using BU's lab approach, researchers successfully predicted the effects of mixtures tested in the laboratory, and work done primarily in collaboration with Dr. Mark Hahn of the BU Superfund Center. In particular, BU scientists tested combinations of compounds that act via the AhR (the "dioxin receptor"), including full agonists (those that achieve maximal effect), partial agonists, and antagonists. The lab is planning to apply their method to other kinds of mixtures, in addition to

developing software that will enable others to more easily apply it. The software will be made freely available via BU's research translation core.

The lab's method for concentration addition has the potential to impact public health and decision-making by increasing the limited stock of tools for assessing chemical mixtures, and by improving the sophistication of risk assessments currently done with the TEF method. The method could find use at Superfund sites and in many other situations. It may have the most direct impact in assessing mixtures of dioxin-like compounds, with likely impacts on the assessment of other endocrine-disrupting compounds.

The interdisciplinary nature of the BU SBRP has been critical to lab's approach to the problem; this work relies on the combined efforts of molecular biologists, statisticians, chemists and epidemiologists.

Trophic Transfer of Toxic Metals in Aquatic Food Webs

Dartmouth College SBRP

Project Leaders: Carol Folt, Ph.D., and Celia Chen, Ph.D.

Research led by Dartmouth scientists found that animals feeding on nutritious, high-quality food have lower concentrations of toxic methylmercury in their tissues. The result suggests ways in which methylmercury—a neurotoxin that can accumulate to hazardous levels—can be slowed in its passage up the food chain to fish.

"This research provides evidence that by eating high-quality food, organisms may reduce their bodily concentration of a contaminant," said lead author Roxanne Karimi, a graduate student in the Dartmouth Department of Biological Sciences. "These findings allow us to predict the conditions under which freshwater fish are likely to carry relatively high mercury levels."

The research is reported in a paper titled "Stoichiometric controls of mercury dilution by growth," published in the *Proceedings of the National Academy of Sciences.*

In laboratory experiments, Karimi and colleagues from Dartmouth, Lakeland College, and Stony Brook University, studied the translucent water flea *Daphnia pulex*, a species of zooplankton that is one of the chief food sources for freshwater fish. Over five days, the team measured the growth of two groups of juvenile *Daphnia*, which, in their mature state, are about 2-3 millimeters in length. Both groups were fed the same amount of algae contaminated with trace amounts of methylmercury; however, one group's algae were of greater nutritional value.

Research demonstrated that those animals that received the nutritious, phosphorous-rich algae grew 3.5 times faster than the other group. Although the faster growing zooplankton ingested roughly the

same amount of methylmercury as the other group, they ended up with one-third the concentration of toxin in their tissues because, as they grew faster, the toxin was diluted.

Methylmercury is a neurotoxin found in all water bodies. While normally present in the water in trace amounts, methylmercury presents a serious health hazard to humans due to biomagnification, a process in which a toxin occurs in higher and higher concentrations in animal tissue as one moves up the food chain. *Daphnia* and other zooplankton are a major source of methylmercury for lake fish. The research suggests that when water fleas and other zooplankton grow rapidly by feeding on high quality food, the rate at which methylmercury is accumulated and transferred through the food chain may decrease.

This same effect could occur in other organisms for other contaminants, such as PCBs and DDT, which are also biomagnified in a food chain, Karimi said. “These contaminants pose health risks because they tend to remain in the body and so accumulate to high concentrations. When organisms have the optimal combination of nutrients available to them, they are able to gain more weight relative to the amount of toxin they get from their food. This is what results in the process of diluting the toxin by rapid growth.”

This study is one of a number of ongoing research projects at Dartmouth that look at methylmercury and other toxic heavy metals in aquatic food webs. Many of these projects are being undertaken by Dartmouth’s Center for Environmental Health Sciences and one of its programs or the Dartmouth Toxic Metals Research Program, which is funded by the Superfund Basic Research Program (SBRP).

“One of the most distinctive aspects of Dartmouth’s center and the SBRP in general is the drawing together of scientists from multiple disciplines to solve problems of significant human impact,” said Karimi’s advisor and co-author Carol Folt, Dartmouth dean of faculty, professor of biological sciences, and associate director of the Dartmouth toxic metals program. “Dartmouth’s program has made great strides in addressing public and environmental impacts of both arsenic and mercury with this approach. Our particular focus—understanding the environmental factors that drive mercury to reach some of the highest levels in fish from the most pristine systems—is of special value for public policy. Mercury in fish is a worldwide issue of concern, meriting region-wide approaches for effective and timely mitigation and global cooperation.”

[Note: The first author and principal researcher on this research paper, Dr. Roxanne Karimi, was a trainee on the Training Core of the Dartmouth SBRP program grant during this research; she recently won the 2007 Karen E. Wetterhahn Memorial Award for this work. Dr. Karimi completed her Ph.D. in June 2007, working with Carol Folt and Celia Chen on Trophic Transfer of Toxic Metals in Aquatic Food Webs, and is now a postdoctoral fellow at SUNY Stony Brook.]

Non-additive Aryl Hydrocarbon Receptor (AhR) Ligand Interactions

Michigan State University SBRP

Project Leader: Timothy R. Zacharewski, Ph.D.

Dioxins and other closely related halogenated aryl hydrocarbons (HAHs) are ubiquitous environmental contaminants that elicit a broad spectrum of toxic effects. Evidence suggests that the toxicity produced by this class of chemicals is due to inappropriate changes in gene expression that are mediated, in part, via the activation of the aryl hydrocarbon receptor (AhR). The objective of this project has been to examine the non-additive responses elicited by mixtures of AhR agonists, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3,3',4,4',5-pentachlorobiphenyl (PCB126), and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153). In order address the responses elicited by mixtures of AhR agonists, quantitative baseline datasets for each individual compound are necessary to facilitate the analysis.

The Michigan State University SBRP lab's research efforts have focused on examining the global gene expression responses elicited by the individual compounds (TCDD, PCB126, PCB153) through the use of custom cDNA microarrays. Comprehensive temporal and dose-response analysis were performed *in vivo,* using mouse models, and *in vitro,* with mouse, rat and human cell lines. cDNA microarray analysis of hepatic tissue from mice treated with 300 μg/kg PCB126 is complete, as is the manuscript accepted for publication (Kopec *et al*., Comparative toxicogenomic examination of the hepatic effects of PCB126 and TCDD in immature, ovariectomized C57BL/6 mice. *Toxicol Sci.* 2007 Nov 27; [Epub ahead of print]). Comparable studies with PCB153 are in progress. Preliminary studies suggest that PCB153 elicits biological activities, which is mediated through the pregnane X receptor/constitutive androstane receptor (PXR/CAR).

Comparative studies have been conducted with immortalized cell-lines derived from mouse (Hepa1c1c7), rat (H4IIE) and human (HepG2) liver(s) treated with 10 nM TCDD. Statistical analysis of the microarray data identified 738, 77 and 986 unique genes that were differentially regulated at one or more points in the mouse, rat and human cell lines, respectively. Comparison of orthologous genes (i.e., genes in different species that are similar to each other because they originated from a common ancestor) revealed that human and mouse cell lines shared the greatest number. In contrast, the fewest overlapping gene expression changes were found between the rat and human; however, the analysis also revealed a significant number of species-specific gene expression responses that may correlate with the differences in toxicity. Additional studies with PCB126, 100 nM indolo(2,3b) carbazol (ICZ) ,and beta-naphthaflavone in Hepa1c1c7 cells are in progress to compare the gene expression profiles between toxic and non-toxic AhR ligands. Further studies with human primary cells have been initiated and presently ongoing.

It is important to emphasize that exposure to PCBs and dioxins rarely occur by way of individual compounds; thus, the effects exerted by these environmental contaminants most often arise from

exposure to multiple HAHs simultaneously in the form of mixtures. ***These current studies provide baseline data for individual AhR ligands that will serve to facilitate the identification of non-additive responses elicited by mixtures of these ligands.***

Tools to Reduce Bioavailability of Toxins

Texas A&M University SBRP

Project Leader: Tim Phillips, Ph.D.

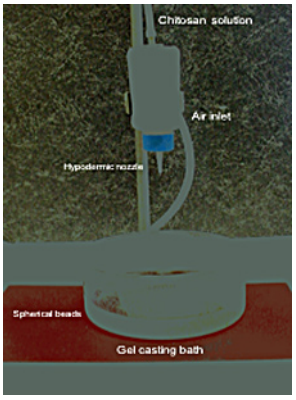

Figure 1a: Preparation (casting) of chitosan gel beads.

The Phillips Laboratory focused on the development of practical chemical strategies for the detoxification of food-borne and environmental carcinogens. Specifically, they have utilized molecular techniques to optimally design materials for the sorption and detoxification of aflatoxins, halogenated aromatic hydrocarbons, and priority metals. Enterosorption involves the use of high affinity, high capacity agents capable of sequestering toxins in the gastrointestinal tract and significantly decreasing their bioavailability, toxicity, and carcinogenicity. Recent research has centered on the remediation of arsenic (As), and the development of an arsenic enterosorbent for therapy in animals and humans at risk for As toxicity. Using a simulated gastrointestinal model, ferrihydrite was found to effectively sorb As(V) and As(III) in the stomach and intestine. Encapsulation of ferrihydrite in chitosan gel beads was shown to enhance As binding. The enterosorption strategy is novel, inexpensive, and should be easily disseminated in a variety of dose forms, including capsules, tablets, and powders. Because of this work, the Office of Technology Commercialization (OTC) at Texas A&M University has launched a Texas-based start-up company (i.e., Texas EnteroSorbents, Inc.). One of the objectives of this new company is to commercialize novel products that will serve to diminish exposure and risk from diverse food-borne and environmental carcinogens.

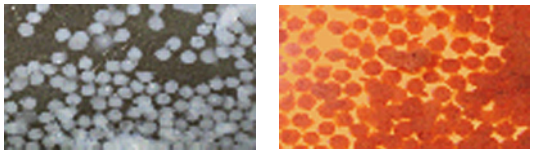

Figure 1b: Left, Chitosan gel beads. **Right,** Chitosan gel beads amended with ferrihydrite.

Toxicogenomic Analysis of Nuclear Xenobiotic Receptors, PXR and CAR, in Chemical Metabolism and Human Health

University of California, San Diego

Project Leader: Ron Evans, Ph.D.

Dr. Ron Evans and his lab study how two nuclear receptors (NR), PXR and CAR, function as principal molecular sensors for the elimination of xenotoxic compounds. This action is mediated through the activation of the hepatic detoxification system, which is comprised of microsomal cytochrome P450 enzymes (CYPs) and other oxidative and hydroxylative enzymes (Phase I); conjugation enzymes, such as glucuronosyl- and sulfo-transferases (Phase II); and membrane-bound drug transporters, such as multiple drug resistance I (MDR1) (Phase III). The lab developed high through-put (HTP) screens for drugs and environmental toxins that activate the xenobiotic response genomic network. These screens can be conducted relatively inexpensively and quantitatively in a variety of cultured cell lines. Currently, researchers have screened several commercial chemical libraries for both human

and rodent PXR/CAR activators (more than 10,000 compounds, including a number of compounds listed on the CERCLA priority list of hazardous substances). In addition, scientists are conducting extensive analysis of genomic response to toxins using the Affymetrix gene chip array along with quantitative polymerase chain reaction (Q-PCR) techniques.

In collaboration with Erin Schuetz (St. Jude Children's Research Hospital), Dr. Evans' team is determining the genomic responses of PXR and CAR target genes in over 250 intestinal samples from human patients. The patient targets in this study are those that underwent duodenal and colonic intestinal biopsies. Patient samples were taken on four separate occasions: before treatment, two days after the treatment (acute), and an additional seven and 14 days (chronic) following the biopsies of four times daily oral xenotoxic drug exposure (rifampin, phenobarbital, phenytoin, carbamazepine or placebo). The laboratory is creating a vital database of the temporal patterns of PXR and CAR regulated gene expression changes in the human intestine – genes that may prove valuable not only to toxicologists, pharmaceutical scientists, and gastroenterologists, but also to the entire scientific research and clinical community.

Rodents and humans differ in their response to xenobiotic toxicants. Using homologous recombination, the lab created strains of mice "humanized" for PXR and CAR. The resulting mice display humanized response traits, and offer a greatly improved screening system for environmental toxins specifically harmful to humans. Scientists are conducting studies to compare rodent and human responses to xenotoxicants, as well as studying mice with humanized xenobiotic sensors.

As a part of the HTP robotically driven assays for xenobiotic compounds that activate the key nuclear receptors, Dr. Evans' lab found that ~5% of approximately 5000 compounds activate human Estrogen Receptor alpha (ERα). The ERα activators are structurally unrelated chemicals, and include anticoagulants, such as warfarin, dicumarol and dihydroxyflavone; antibacterial drugs, such as minocycline hydrochloride, mafcilin, trimethoprim and sulfamethoxazole; and anti-inflammatory drugs, such as sulindac and mefenamic acid. Additionally, certain environmental polycyclic aromatic hydrocarbons are also ERα activators; these compounds, collectively referred to as xenogenic estrogens, have been proposed to contribute to endocrine disruption syndromes in humans. While the basis for endocrine disruption is unclear, collaborator Dr. Vincent Giguere depicted that the estrogen receptor directly binds the promoters of cytochrome P450 genes involved in xenobiotic and steroid hormone metabolism. These observations suggest that the estrogen receptor may act as an atypical promoter of xenobiotic clearance.

NRs are generally believed to be activated by ligand induced release of co-repressor, followed by the association of a co-activator. This mechanism has been poorly explored for CAR. Scientists find that the overexpression of the NR co-repressor "silencing mediator of retinoid and thyroid receptors" (SMRT) strongly inhibits CAR transactivation of the CYP2B promoter. Using *in vitro* and *in vivo* assays, scientists identified a direct interaction between the ligand-binding domain of CAR

and SMRT that is dependent on a few critical amino acid residues in the SMRT nuclear receptor-interaction domain (RID). Most importantly, the lab found that a mouse strain harboring targeted knock-in point mutations in the SMRT RID that researchers created shows a spontaneous increase in the expression of CAR target genes; as a result, these mutant mice have a higher level of drug metabolism, resulting in increased toxicity resistance when challenged with multiple chemicals. Taken together, Dr. Evans and his team propose that SMRT may act as a new key regulator of hepatic drug metabolism.

The Role of Oxidative Stress in Toxicity and Carcinogenicity

University of North Carolina, Chapel Hill SBRP
Project Director: J.A. Swenberg, Ph.D., D.V.M.

One of the focuses of Dr. Swenberg's research is the use of chicken B lymphocyte (DT40) cells to identify critical pathways of DNA repair for chemicals relevant to the EPA Superfund program. DNA damage response and repair have a major impact on human health, including cancer, age-related diseases, and various inherited disorders. In addition to yeast and mouse models, DT40 cells have been utilized for genetic studies to better understand the function of genes related to human diseases for approximately two decades. DT40 cells, transformed with an avian leukosis virus, have been used to generate a series of isogenic knockout DT40 mutants (1). The targeted integration in DT40 cells occurs at essentially all loci in these cells with efficiencies that are orders of magnitude higher than those observed in mammalian cells.

In collaboration with Shunichi Takeda (Kyoto University, Japan) and many other DT40 researchers, the University of North Carolina, Chapel Hill (UNC-CH) SBRP laboratory successfully set up a DT40-based DNA damage response analysis system with a series of isogenic DT40 mutants to characterize the potential impact of Superfund chemicals on human health. Using this system, Nakamura and colleagues investigated DNA damage responses to formaldehyde (2). Formaldehyde, the smallest aliphatic monoaldehyde, is a major Superfund chemical, and a highly reactive environmental animal and human carcinogen. The general population is typically exposed to formaldehyde through inhalation and ingestion. It has been well-documented that DNA-protein crosslinks (DPC) likely play an important role with regard to the genotoxicity and carcinogenicity of formaldehyde; however, little is known about which DNA damage response pathways are essential for cells to counteract formaldehyde. The UNC lab first assessed the DNA damage response to formaldehyde using chicken DT40 cells with targeted mutations in various DNA repair genes. Scientists showed that hypersensitivity in cell survival to formaldehyde is detected in DT40 mutants deficient in the BRCA/Fanconi anemia (FANC) pathway, homologous recombination, or translesion DNA synthesis. For example, the 50% lethal dose (LD50) by formaldehyde exposure in FANC complementation group D2 (FANCD2)-deficient DT40 cells is approximately 10 times lower than that in parental DT40 cells. In addition, human cells deficient in FANCC and FANCG are also hypersensitive to formaldehyde. These results indicate that the BRCA/FANC pathway is essential to counteract DPCs caused by aliphatic monoaldehydes.

Based on the results obtained from this study (which were recently published in *Cancer Research*), the UNC SBRP lab is proposing that FANC patients could represent a human population hypersensitive to formaldehyde in the environment. Clearly this powerful isogenic cell model system provides valuable information, such as: (i) the existence of possible human populations susceptible to specific environmental chemicals as described above; (ii) the types of biomarkers needed for risk assessment (e.g., formaldehyde-specific DPCs and mutations, in the case of formaldehyde); and (iii) suggests possible mouse models that could be utilized to assess human risk for specific chemicals (e.g., FANC complementation group-knockout mice). Formaldehyde is just one example; researchers are currently applying this system to many other metabolites of Superfund chemicals, including naphthalene quinoid metabolites and benzoquinone.

Sensing Superfund Chemicals with Recombinant Systems

University of Kentucky

Project Leader: Sylvia Daunert, Ph.D.

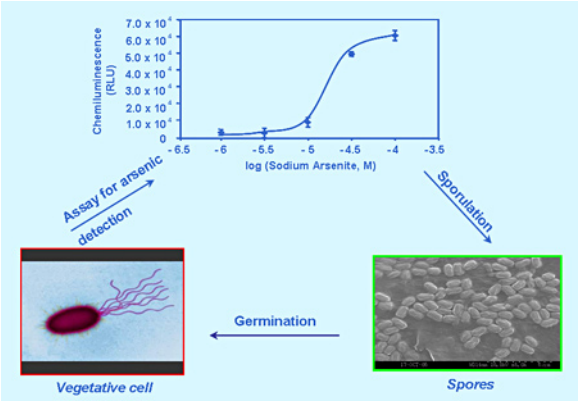

Figure 1: Schematic representation of sensing-sporulation-germination cycles using spore-based sensing systems.

A variety of conventional analytical methods for the detection of environmental pollutants are available; however, these methods are time-consuming, require extensive sample preparation and complex instrumentation, and have low throughput. The main goal of Dr. Sylvia Daunert's research is to develop biosensing systems that are sensitive, selective, rapid, easy-to-use, and amenable to field applications. These methods can be used to quickly screen samples for the presence of pollutants, so that the apprpriate samples can be efficiently selected for further analysis. A strategy for the development of biosensing systems involves the design and preparation of bacterial whole cells and proteins that are genetically engineered to respond to the analytes of interest with the production of a detectable signal (e.g., fluorescence, bioluminescence, chemiluminescence). Dr. Daunert's lab is also working to develop enabling technologies for on-site studies, which aim at the long-term preservation and storage of these biosensing systems, and their incorporation into portable miniaturized analytical devices.

To that end, Dr. Daunert's lab developed whole-cell sensing systems for chlorocatechols, arsenic, biphenyls, hydroxylated PCBs, and dihydroxylated PCBs. Recently, they were able to detect hydroxylated PCBs in trace amounts in whole serum samples, directly, without sample pre-treatment. Dr. Daunert envisions that the method developed can be employed as a rapid and sensitive tool, both, to monitor OH-PCBs in laboratory toxicological studies and to evaluate the presence of bioavailable OH-PCBs in natural environments. The potential of whole-cell sensing systems could be enhanced if they could be used effectively in the field. For that, the lab developed an inexpensive and simple method of preservation, storage, and transport of these sensing systems, which is based on the use

of spore-forming bacteria, such as *Bacillus* species, and exploits the spores' ruggedness. In fact, spores can be very resistant to environmental conditions and shocks, and can easily germinated to generate metabolically active cells. To that end, researchers proved the viability and wide-range applicability of this approach by developing several spore-based whole-cell sensing systems for various analytes, such as arsenic and zinc, and, demonstrated their stability in terms of analytical performance after three cycles of sporulation/germination, as well as after long-term storage (6-8 months) at room temperature. Figure 1 depicts a schematic of the spore-based approach.

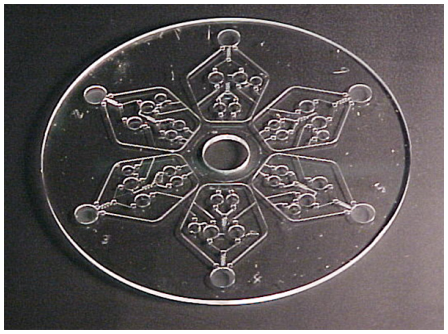

Figure 2: CD with multiple microfluidic architectures.

In order to further enhance the on-site employment of spore-based whole-cell sensing systems, scientists incorporated them into a microcentrifugal microfluidic platform made of poly(methyl methacrylate) (PMMA), in the shape of a compact disk (CD). Figure 2 shows an example of the CD platform with multiple microfluidic architectures. This platform can be easily transported and operated *in situ* using a typical CD reader. Upon demonstration of fast spore germination and bacterial cell growth in the CD miniaturized microfluidic platform, sensing was successfully performed in the CD. This allowed for the rapid detection of analytes in times of the order of minutes, at low μM levels in low μL-volume samples. A different and novel approach to the development of biosensing systems takes advantage of the conformational changes that regulatory proteins undergo upon binding their specific ligands. A series of proteins that bind hydroxylated PCBs have been expressed and purified to develop protein-based sensing systems for hydroxylated PCBs. These proteins are either labeled with various fluorophores or genetically fused to fluorescent proteins and expressed as fusion proteins. Preliminary results obtained with protein-based sensing systems showed faster response and better sensitivity when compared to whole-cell biosensors. Proteins are also amenable to incorporation into microfluidic platforms.

Research Translation Participation at KRCEE Symposium

University of Kentucky

Project/Core Leader: Lindell Ormsbee, P.E., Ph.D.

On October 30-31, 2007, a number of UK-SBRP faculty, students, and post-doc researchers made presentations at the Kentucky Research Consortium for Energy and the Environment (KRCEE) Scientific and Technical Symposium in Lexington, KY. This symposium provided progress toward partnering with representatives from state and federal government, industry, and academia to discuss projects underway at the Paducah Gaseous Diffusion Plant Superfund site in western Kentucky. Government attendees included Environmental Protection Agency, Department of Energy, Kentucky Natural Resources and Environmental Protection Cabinet (EPPC), Kentucky Division of Waste Management, Kentucky Division of Water, Kentucky Geological Survey, Kentucky Cabinet for Health and Family Services, and EPPC Superfund Branch. Sessions covered data management issues, new technologies, historic and current seismic activities and assessments, surface water and ecological issues, and groundwater modeling.

As KRCEE Director, UK-SBRP Research Translation Core Leader Dr. Lindell Ormsbee hosted the event and presented a number of topics, including a land acquisition study. UK-SBRP Chloro-Organic Degradation project leader, Dr. Dibakar Bhattacharrya, and Whole-Cell Sensing project leader, Dr. Sylvia Daunert, presented their research and findings to an audience that included representatives of the US Department of Energy, the US Environmental Protection Agency, the Kentucky Division of Waste Management, and Kentucky and Illinois colleges and universities, as well as technology corporations and consultants. Students supported by the SBRP assisted Dr. Bhattacharrya in his presentation.

A luncheon during the first day of the symposium provided a venue for poster presentations by a number of UK-SBRP graduate students and post-doctoral scholars. From Dr. Bernhard Hennig's group, students presented an overview of the UK-SBRP and a poster entitled "Dietary Flavanoids Block PCB-Induced Proinflammatory Responses in Vascular Endothelial Cells." Dr. Daunert's students presented "Development of a Protein-Based Sensing System for the Quantitation of Hydroxylated Polychlorinated Biphenyls." Dr. Bhattacharrya's students presented "Reductive Dechlorination of 3,3',4,4'-Tetrachlorobiphenyl (PCB77) Using Palladium or Palladium/Iron Nanoparticles and Assessment of the Reduction in Toxic Potency in Vascular Endothelial Cells." The Outreach Core presented "Nutrition Education Provides Outreach to Improve Health-Related Behaviors for Superfund Communities."

In addition to the symposium, the UK-SBRP Research Translation and Training Cores are progressing toward conducting technology transfer by collaborating on a joint communication training/research endeavor for the program's graduate students and postdoctoral scholars. Based on an

extended review of relevant tailored communication and public speaking literature, this project aims to improve the research translation skills of biomedical and engineering graduate students. Pre- and post-test assessments of subject attitudes regarding their own communicative competencies, along with peer and reviewer evaluations of subject presentations, will identify best practices for tailoring messages about complex research processes and outcomes to meet the varied needs and message reception competencies of specific audiences. Pre-testing in December will provide necessary data for a needs assessment that will inform curriculum development.

Finally, the Research Translation Core has provided great strides in their progress toward communicating with broad audiences via the web by assuming management of the UK-SBRP website, and launching a new design that not only highlights internal projects, cores, and accomplishments, but also provides live RSS feeds of international headlines related to those cores and projects. The revitalized website also promotes graduate student and postdoctoral scholar professional development through the posting of detailed information, photographs, and curriculum vitas. Project and core leaders have been given more autonomy in selecting content for their respective pages. In connection with the main SBRP website, the Research Translation Core has also begun working on an interactive website that will be adaptive to the particular type of user (e.g., general public, academia, government, etc.). The design draws upon previous research that indicates better learning and retention rates through tailored messages and computer modules. The site's construction marks a collaborative effort between UK-SBRP RTC personnel and IT staff with the UK College of Communications and Information Studies, which, in 2004, was ranked sixth (nationally) among health communication doctoral programs, and has developed a significant NIH funding record for its health communications research and outreach.

Website completion is anticipated for the late-spring/early-summer of 2008.

NIEHS Environmental Health Sciences Data Resource Portal

University of California, San Diego

Core Leader: Keith Pezzoli, Ph.D.

The Environmental Health Perspectives April 2007 edition (Vol. 115, No. 4) has a research article that describes outreach led by UCSD's SBRP in partnership with Duke University's SBRP, among others. The article, "The NIEHS Environmental Health Sciences Data Resource Portal: Placing Advanced Technologies in Service to Vulnerable Communities," documents the devastating effects of the Katrina-Rita hurricanes that ripped across the Gulf Coast during 2005. It also documents the subsequent efforts of the NIEHS to facilitate the region's recovery. UCSD's Community Outreach Core organized and led the article's research and writing team—a multidisciplinary group of eight co-authors representing a diverse mix of programs, departments and centers (UCSD: Urban Studies and Planning Program; Department of Pharmacology, and Chemistry & Biochemistry; San Diego Supercomputer Center; National Center for Microscopy and Imaging Research; Center for Research

in Biological Systems; and the Department of Neurosciences; DUKE University: Nicholas School of the Environment and Earth Sciences; and the NIEHS). In the same EHP issue (April 2007), the editors drew special attention to the article by highlighting it in the journal's Science Selections.

The effects of Hurricanes Katrina and Rita were severe—the human and environmental health impacts on New Orleans and other Gulf Coast communities will be felt for many decades. The Federal Emergency Management Agency (FEMA) estimates that Katrina's destruction (exacerbated by the chronic loss of wetlands, land subsidence, and inadequate infrastructure) disrupted the lives of roughly 650,000 Americans. Over thirteen hundred people died. The projected economic costs for recovery and reconstruction are unprecedented—in excess of $125 billion. In this context, the EHP article reviews the NIEHS portal's potential as a system to provide decision makers with data, information and tools necessary to: 1) monitor human and environmental health impacts of disasters; 2) assess and reduce human exposures to contaminants; and 3) develop science-based remediation, rebuilding, and repopulation strategies.

The EHP article documents a number of worrisome regional and global trends in coastal urbanization, disaster, and risk. The 2005 Gulf Coast hurricane devastation may be a harbinger of worse things to come. Analysis of this sort constitutes a new frontier in multidisciplinary research and writing. The EHP article's analytic approach bridges the environmental health sciences with urban studies and planning, cyber-infrastructure, community participation, and geographic information systems.

The density of industrial operations in New Orleans and the Mississippi Delta prior to the Katrina disaster, combined with the release of contaminants into floodwaters and their transport throughout urbanized areas, gave rise to many significant environmental health concerns. At the same time, the flooding of thousands of homes and over a hundred schools spurred indoor growth of mold and other bioaeroallergens that raised concerns about human respiratory and neurological health. All of these potential exposures took place within the context of high psychosocial stress and, for many, low socioeconomic status – conditions which often exacerbate the effects of adverse environmental exposures. The scale and complexity of the ensuing environmental health issues made tracking environmental hazards, and focusing medical and environmental response and recovery resources in areas of greatest need, an obvious priority. The EHP article examines these issues/challenges from an integrated perspective.

Specifically, the co-authors articulate an analytic framework that takes into account the rising magnitude of disasters and vulnerability on a global scale, the emergence of new modes of knowledge production and networking, the increasing appreciation of community-linked research, and advances in exposure biology and gene-environment interaction studies.

The NIEHS portal described in the EHP article was designed as a prototype for environmental health science research in the modern scientific and computing era. In taking on such a challenge, the co-

authors of the EHP article argue that advances in technology and the availability of powerful tools alone will not suffice if a culture and incentives for multidisciplinary collaboration are not in place, and systems are not built with user needs in mind.

Research Translation Core Hosts Workshop on Environmental Health Issues

Iowa Superfund Basic Research Program

Project Leader: David Osterberg, Ph.D.

With sponsorship from the Superfund Basic Research Program, the Environmental Health Sciences Research Center (EHSRC) and the National Conference of State Legislatures (NCSL) presented a workshop, "Environmental Health Issues in the Midwest: A Two-Day Workshop for Midwest State Legislators" in Iowa City, Iowa on October 21-23, 2007. The overarching goal of the workshop was to translate environmental health research into knowledge that is important to Midwestern legislators. Four environmental health topics most relevant to the scientific mission of the EHSRC and the Iowa Superfund Basic Research Program (ISBRP), all of which relate to environmental health in the Midwest, were addressed: 1) polychlorinated biphenyl (PCB) contamination in the Great Lakes region, 2) the health effects of mercury and its reduction through increased renewable energy production, 3) radon incidence and mitigation, and 4) the environmental effects of Confined Animal Feeding Operations. The workshop was attended by 10 legislators and four legislative staff members from the states of Iowa, Minnesota, Nebraska, Illinois, Wisconsin and Missouri; four staff members from EPA Regions 5 and 7; eight members of the EHSRC's Community Outreach and Education Stakeholder Advisory Board; three staff from Iowa environmental agencies, including the director of the Governor's recently established Iowa Office of Energy Independence; expert researchers from the ISBRP, Environmental Health graduate students; and other interested participants. The ISBRP Outreach Core and Research Translation Core organized the Workshop, which had informational panels and interactive sessions.

In the laboratory, ISBRP researchers were participating in broad efforts that extended among several projects and cores, namely a study of the expression of xenobiotic-metabolizing and antioxidant enzymes induced by PCBs and metabolites. Synthetic PCBs and metabolites selected for these studies, included those that activate (or suppress) transcription factors, such as Ah, CAR, PXR, and PPARα. Those rats exposed to these highly pure and well-characterized xenobiotics showed changes in redox and antioxidant status (notably in glutathione oxidation state and levels), altered activities of antioxidants (e.g., glutathione peroxidase), and xenobiotic-metabolizing enzymes (e.g., cytochromes P-450 and sulfotransferases). These data represent cooperation of Superfund researchers in three projects (Projects 1-3) and three research support cores (Synthesis, Analytical and Inhalation Toxicology), and have broad implications on human health. These studies complement on-going research in Project 2 (Oxidative Stress) and in Project 3 (Studies of Direct Interactions of PCBs and their Hydroxylated Metabolites on sulfotransferases, especially their Roles as Substrates and Inhibitors). During studies on rat family 1 enzyme, SULT1A1, Superfund

researchers discovered that partial oxidation of the cysteine residues alter catalytic function such that several OHPCBs that inhibit the enzyme under fully reduced conditions become substrates for sulfation when the enzyme is partially oxidized. This strongly suggests that oxidative stress (a major focus of Projects 1 and 2) may alter the metabolism of some OHPCBs through sulfation catalyzed by SULT1A1. These exciting findings at the intersection of metabolism, oxidative stress and enzyme induction will be a focus of investigation during the upcoming year.