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Health Effects

**Environmental phthalate- and PPAR-mediated toxicity in the developing immune system**
Boston University SBRP
Project Leaders: Jennifer Schlezinger and David Sherr

When a living cell encounters a foreign chemical a variety of things can happen. Depending on the amount of chemical, the most frequent response is that nothing of consequence happens. However we know that environmental chemicals can also turn a normal cell into a cancer cell (carcinogenesis), interfere with the proper function of the cell (toxicity), or kill the cell. Cell death can either be from an outright destruction of vital cellular processes or because the chemical tricks the cell into committing suicide (apoptosis, programmed cell death). The suicide program is a normal process, needed to protect the body from cells that have been altered in ways that might hurt the whole organism (e.g., cancer cells) or during the normal developmental changes that occur as a body grows and changes shape. This research is unraveling the ways that two very common environmental chemicals – one, by-products of combustion (PAHs), the other a commonly used plasticizers (phthalates) – cause an important cell of the immune system, the B-lymphocyte which makes antibodies, to initiate a suicide program and hence die.

In particular, this project is examining the way that the chemicals interact with a chain of events that starts with the stimulation of a signaling button on the surface of the cell called PPAR. Research is showing that although the details of how chemicals cause programmed cell death differ, they require similar cellular components, called caspases, to make it happen. Drs. Schlezinger and Sherr have identified that one particular caspase called caspase-9, is required in all three otherwise disparate cell death routines related to PPAR. This is a significant finding that may show how B cells can be sensitized to death by the activation of a caspase feedback-loop initiated by multiple kinds of different pollutants.

**Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: Baseline results from the Health Effects of Arsenic Longitudinal Study (HEALS)**
Columbia University SBRP
Project Leader: Habibul Ahsan

Millions of people in the world, including more than 3 million in the United States and more than 50 million in Bangladesh, are chronically exposed to arsenic (As) – a potent human carcinogen. However, our knowledge about the health effects of arsenic exposure at doses <100 µg/L is primarily based on extrapolations from high-dose studies. Most of the studies conducted to date, including cohort studies, have employed retrospective ecological exposure measurements in their dose-response analyses, making individual-level exposure assessment extremely difficult. Using data from nearly 12,000 men and women in Araihazar, Bangladesh, Dr. Ahsan’s group investigated the effects of arsenic exposure on arsenic-induced skin lesions, a hallmark of arsenic poisoning, for doses ranging from very high to very low measured at the individual level. They also evaluated the influence of key host factors on this association, including body mass index (BMI), gender, and age. Measures of arsenic exposure include urinary creatinine-adjusted arsenic concentration and a time-weighted well water arsenic concentration (TWA) incorporating the information on well use history. Adjusted prevalence odds ratios (PORs), excess relative risk (ERR), and relative excess risk for interaction (RERI) were estimated to assess the main effects of arsenic and its interaction with key host characteristics (BMI, male gender, and age).
Compared to persons drinking water containing <8.1 µg/L of arsenic, adjusted PORs of skin lesions for those drinking water with 8.1-40.0, 40.1-91.0, 91.1-175.0, and 175.1-864.0 µg/L of arsenic were 1.91 (95% CI: 1.26-2.89), 3.03 (95% CI: 2.05-4.50), 3.71 (95% CI: 2.53-5.44), and 5.39 (95% CI: 3.69-7.86), respectively. While creatinine-adjusted urinary arsenic does not directly correspond to TWA categories, the elevated risk was statistically significant also for the second lowest category. In linear dose-response analyses, Dr. Ahsan estimated that a 10 µg/L increase in tube well water arsenic concentration was associated with an ERR of 0.122 (95% CI: 0.087-0.171), i.e., those exposed to arsenic doses of 10 µg/L had a 1.22 times higher risk of developing skin lesions compared to those with zero dose.

Males appeared to be disproportionately more susceptible to skin lesions than females at higher levels of TWA. The RERI estimates for the interaction of male gender and top four TWA quintiles were 2.7 (95% CI: -0.0, 5.4), 5.9 (95% CI: 0.8-10.9), 8.9 (95% CI: 1.7-16.1), and 11.6 (95% CI: 2.2-21.0). The corresponding attributable proportion due to interaction (AP = RERI/POR for joint exposures) is 39%, 52%, 64%, and 61%, which corresponds to the fraction of skin lesion risk among males with each higher level of arsenic exposure that can be attributable to the synergistic effect of male gender and arsenic. At each level of TWA, older participants were more susceptible to skin lesions than their younger counterparts. At each level of TWA, we also observed a trend for the adjusted PORs for skin lesions to be higher for participants with lower levels of BMI.

This study reports a strong dose-response effect of arsenic exposure on skin lesion risk in Bangladesh. This dose-response effect was uniformly evident in several statistical models appropriate for analyzing cross-sectional data. Dr. Ahsan’s group found that well water arsenic concentrations <50 µg/L (the currently permissible limit in Bangladesh and other countries and in the US until very recently) is associated with an increased risk for skin lesions. Previous studies in other countries, including those in Bangladesh and West Bengal, had failed to show any increased risk at the lower arsenic dose range, partly because those studies lacked sufficient sample size at the low-level arsenic exposure. The researchers found that male, older, and/or thinner participants were more likely to be affected by arsenic exposure. These findings need to be taken into consideration for risk assessment and policy-making decisions.

**Folate deficiency, hyperhomocysteinemia and hypomethylation of genomic DNA are risk factors for arsenic-induced skin lesions: A nested case-control study in Bangladesh**

Columbia University SBRP
Project Leader: Mary V. Gamble

Chronic arsenic (As) exposure currently affects more than 100 million people worldwide. In Bangladesh alone, this exposure is affecting approximately 50 million residents. Individuals exposed to arsenic are at increased risk for cancers of the skin, bladder, lung, and liver. Non-cancer health outcomes include stroke, ischemic heart disease, and neurologic consequences. Dr. Mary Gamble is conducting a study focused on premalignant arsenic -induced skin lesions.

Methylation of ingested inorganic arsenic (InAs) to methylarsonic (MMA) and dimethylarsinic acids (DMA) relies on folate-dependent one carbon metabolism. DMA has a shorter circulating half-life than other arsenic metabolites, and is readily excreted in urine. Therefore, methylation of arsenic facilitates excretion of arsenic in urine. There is considerable inter-individual variability in arsenic methylation, and mounting evidence indicates that nutritional status influences this variability. For example, Dr. Gamble previously found that there is a very high prevalence of hyperhomocysteinemia in Ban-
gladesh and that folate deficiency and hyperhomocysteinemia are associated with a reduced capacity to methylate arsenic. In a recent, randomized, controlled trial, her group discovered that folic acid supplementation resulted in an increase in the proportion of total urinary arsenic excreted as DMA and a reduction in MMA and InAs. To begin to assess the potential impact on health outcomes, the researchers tested the hypothesis that low plasma folate and/or hyperhomocysteinemia are risk factors for subsequent development of arsenic-induced skin lesions (SLs). Because folate and homocysteine influence DNA methylation, and previous studies employing cell culture and animal models indicate that arsenic exposure also influences DNA methylation, the researchers also investigated whether genomic hypomethylation of peripheral blood leukocyte DNA (PBL DNA) is a risk factor for SLs.

Dr. Gamble’s group conducted a nested case-control study in Araihazar, Bangladesh. The study participants were a subset of the 11,746 men and women of the Health Effects of Arsenic Longitudinal Study (HEALS) cohort study between the ages of 18 and 65 years who were recruited between October 2000 and May 2002, and who continue to be followed at two year intervals. A total of 316 incident SL cases (i.e. people who were free of SLs at baseline, but developed SLs by 2 years after recruitment) and 316 controls were individually matched for gender and age (within 5 years), and frequency matched for water arsenic (within 100 µg/L). Folate, homocysteine and DNA methylation were assessed in samples that had been collected at the baseline visit, at which time all participants were free of SLs. Genomic methylation of PBL DNA was determined using a [3H]-methyl incorporation assay.

Conditional logistic regression analyses found the odds ratios (95% C.I.s) for subsequent development of SLs for participants who, at baseline, had either marginal folate status (plasma folate < 9 nmol/L), high Hcys (> 10.4 µmol/L), or hypomethylated PBL DNA (below the median) were 1.8 (1.21 – 2.81; p = 0.005), 1.68 (1.2 - 2.5; p = 0.01), and 1.8 (1.17 – 2.80; p = 0.008), respectively. The significance of these results was not altered after further adjustment for covariates.

Folate deficiency, hyperhomocysteinemia, and hypomethylation of genomic PBL-DNA appear to be independent risk factors for arsenic-induced skin lesions. Given that there is a very high prevalence of hyperhomocysteinemia in Bangladesh, particularly among males, a careful assessment of the risks and benefits of a country-wide folic acid intervention is warranted.

Folic acid supplementation lowers blood arsenic by increasing arsenic methylation
Columbia University SBRP
Project Leader: Mary V. Gamble

Chronic arsenic (As) exposure currently affects more than 100 million people worldwide. Methylation of ingested inorganic arsenic (InAs) to methylarsonic- (MMA) and dimethylarsinic acids (DMA) relies on folate-dependent one carbon metabolism. In a recent, randomized, controlled trial, Dr. Gamble’s research group analyzed total arsenic and arsenic metabolites in urine and showed that folic acid supplementation resulted in an increase in the proportion of total urinary arsenic excreted as DMA and a reduction in MMA and InAs. Because DMA has a shorter circulating half-life than other arsenic metabolites, Dr. Gamble hypothesized that facilitation of arsenic methylation via folic acid supplementation might lower total blood arsenic (tbAs) concentrations. Methodological advances, using inductively coupled mass spectrometry with a dynamic reaction cell (ICP-MS-DRC) permitted the researchers to test this hypothesis by measuring total arsenic and arsenic metabolites in blood in 130 participants with low plasma folate (< 9 nmol/L) before and after 12 weeks of supplementation with folic acid (400 µg/d) or placebo. These methodological advances have also led to the discovery that blood arsenic is a biomarker of
arsenic exposure that is directly associated with the risk for arsenic-induced skin lesions. Thus, lowering blood arsenic with folic acid could have therapeutic potential to reduce the risk for arsenic-induced illnesses.

Folic acid supplementation reduced MMA in blood (bMMA) by 22.2 ± 2.9 percent (mean ± SE) as compared to placebo (1.24 ± 3.0 percent, p < 0.0001). On average, bMMA was reduced from 4.1 ± 2.6 µg/L pre-intervention to 3.0 ± 1.8 µg/L post intervention (mean ± SD) (p < 0.0001) for the folic acid group. For the placebo group, corresponding values were 4.0 ± 2.5 vs. 3.8 ± 2.3 µg/L (p = 0.21). Eighty-five percent of all participants in the folic acid group experienced a decline in bMMA; 55% of participants in the placebo group experienced a decline in bMMA. Greater declines were observed for individuals who had higher bMMA at baseline than for those that had lower bMMA at baseline. There was no change in DMA in blood; the latter is rapidly excreted in urine as was evidenced by an increase in urinary DMA (p = 0.01). InAs in blood decreased by 18.5% for the folic acid group vs. 10.6% for the placebo group (p = 0.07). Folic acid supplementation reduced tbAs by 14% vs. 2.5% for the placebo group (p = 0.02). Pre- vs. post-intervention tbAs concentrations declined from an average of 9.9 ± 5.1 to 8.2 ± 4.2 µg/L (P < 0.0001) for the folic acid group, whereas the placebo group had a modest, nonsignificant decline from 9.6 ± 5.0 to 9.1 ± 4.9 µg/L (P = 0.2). The data were similar with further adjustment for age, BMI, and gender.

Population studies indicate that individuals with relatively greater portions of MMA and smaller proportions of DMA in urine are at greater risk for skin lesions, skin and bladder cancer, and peripheral vascular disease. Furthermore, studies employing cell culture and animal models suggest that MMAIII may be the most cytotoxic and genotoxic arsenic intermediate. The current study indicates that folic acid supplementation lowers total blood arsenic primarily by lowering concentrations of MMA in blood. These findings imply that folic acid supplementation may reduce body stores of arsenic. In conclusion, therapeutic strategies to facilitate arsenic methylation, particularly in populations with a high prevalence of folate deficiency and/or hyperhomocysteinemia such as Bangladesh, may lower blood arsenic concentrations, and thereby contribute to the prevention of arsenic-induced illnesses.

The role of arsenic in melanoma treatment
Columbia University SBRP
Project Leader: Tom Hei

The incidence of melanoma has substantially increased worldwide over the last 40 years. Although melanoma accounts for only 10% of the skin cancers, it is responsible for at least 80% of the mortality cases. Approximately 8,000 Americans died of melanoma in 2005 and 62,200 new cases of melanoma were diagnosed in 2006. Advanced melanomas are highly refractory to conventional radio- and chemotherapy and no effective therapy exists to inhibit metastatic spread of this cancer.

Arsenite is a two-sided sword. On one hand, it is a well established human carcinogen and, on the other hand, it has been used successfully in the treatment of acute promyelocytic leukemia by inducing apoptosis. Dr. Tom Hei previously showed that only ~20% of human melanoma cell lines are conducive to apoptosis by arsenite treatment alone. A recent clinical trial in the use of single regimen of arsenite trioxide in the treatment of metastatic melanoma fails to produce any significant improvement in clinical outcome. These findings suggest that multi-modality regimen is required in the design of treatment strategy.

The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) has been shown to have great anti-tumor potential via induction of apoptosis in a wide variety of human TRAIL-Receptor-1 (TRAIL-R1/DR4) and Receptor-2 (TRAIL-R2/DR5) -positive cancer cells including melanomas. In contrast, most normal human cells often contain high levels of
decoy receptors TRAIL-R3 and TRAIL-R4 on cell surface, thus, preventing induction of apoptotic signaling. Many metastatic tumors, including melanomas, acquired the secondary resistance to TRAIL-mediated apoptotic signaling (almost 60% of tumor cell lines are resistant to recombinant TRAIL). Besides increasing the number of decoy receptors, such resistance may occur at different points of the TRAIL-induced death signaling pathway. Suppression of surface expression of the death receptors (TRAIL-R1/DR4 and TRAIL-R2/DR5), dysfunction of death receptors due to mutations, suppression of the pro-apoptosis caspase-8 or caspase-3 functions, over-expression of inhibitors of apoptosis, such as cFLIP (cellular FLICE inhibitory protein), XIAP (X-linked inhibitor of apoptosis), Bcl-2 and Bcl-xL directly correlates with resistance to TRAIL in many types of cancer. Especially, progression in melanoma is often linked with decreased surface expression of TRAIL-R1/R2. On the other hand, general activation of the NF-κB, PI3K-AKT and BRAF-MEK-ERK signaling pathways, which are characteristic features of advanced tumors, including metastatic melanomas is connected with protection against TRAIL-mediated apoptosis via up-regulation of gene expression of anti-apoptotic proteins.

Dr. Hei’s previous studies demonstrated that sodium arsenate may dramatically affect signaling pathways in melanoma cells and differentially regulate AP-1/cJun, NF-κB and STAT3 transcription factor activities that control expression of numerous genes for proliferation, survival and apoptosis. Sodium arsenite is known to suppress both the IKK-NF-κB and JAK2-STAT3 signaling pathways and to activate the MAPK/JNK-c-Jun pathways, thereby committing some cancers to undergo apoptosis. In our recent studies, we found sodium arsenite treatment up-regulated TRAIL-mediated apoptosis in human and mouse melanomas by increasing surface levels of death receptors, TRAIL-R1 and TRAIL-R2, through increased translocation of these proteins from cytoplasm to the cell surface. In addition, activation of c-Jun and suppression of NF-κB by sodium arsenite resulted in upregulation of the endogenous TRAIL- and down-regulation of the cFLIP gene expression (which encodes one of the main anti-apoptotic proteins in melanomas). This results in cFLIP protein degradation and acceleration of TRAIL-induced apoptosis. Similar effects can also be achieved using RNAi to suppress cFLIP expression. In contrast, suppression of cyclooxygenase-2 (COX-2) by RNAi or NS398 substantially increased levels of both TRAIL-induced and arsenite-induced apoptosis. Finally, over-activation of the protein kinase AKT increased melanoma survival in cell culture and dramatically accelerated growth of melanoma transplant in vivo, highlighting a role of AKT suppression for effective anti-cancer treatment.

A better understanding in the apoptotic signaling pathways induced by arsenic treatment in melanoma cells with concurrent modulator of pro-apoptotic and suppression of anti-apoptotic pathways will provide a useful mechanistic rationale for effective treatment design for this often fatal cancer.

Arsenic as an endocrine disruptor: multiple steroid receptors
Dartmouth College SBRP
Joshua Hamilton

Dartmouth Medical School investigators are learning more about how low doses of arsenic, such as the levels found in drinking water in many areas of the United States, affect human physiology. In a paper published online on Dec. 2 in the journal Chemical Research in Toxicology, the researchers report that three different steroid hormones all show similar responses to arsenic, suggesting a broader effect and a common mechanism of arsenic on how these hormones function.

“Since most of the health consequences of exposure to arsenic - various cancers, diabetes, heart and vascular disease, repro-
ductive and developmental effects, etc. - involve these same steroid receptors, we think that disruption of their normal function could explain, in large part, how arsenic can influence so many disease risks,” 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.

Hamilton’s laboratory had earlier found that arsenic disrupts the activity of the glucocorticoid receptor, and this follow up study considered the progesterone and mineralocorticoid receptors, which regulate a wide range of biological processes. This work was done in collaboration with Jack Bodwell, the lead author on this paper and a research associate professor of physiology at Dartmouth Medical School.

Hamilton, Bodwell, and their team found that arsenic appears to suppress the ability of all three of these critical receptors to respond to their normal hormone signals. Chemicals that disrupt steroid hormone receptor signaling are called endocrine disruptors, and this study provides further evidence that arsenic, a metal, does not behave like other endocrine disruptors such as pesticides.

“Arsenic does not activate these receptors, as some endocrine disruptors do, by mimicking the natural hormone, nor does it block the ability of the normal hormones to activate their specific receptor, as most other endocrine disruptors do,” says Hamilton, who is also a professor of pharmacology and toxicology at Dartmouth Medical School. “Nor does it affect the ability of the hormone-activated receptor to move to the nucleus of the cell or to bind to DNA to initiate gene expression. Yet, somehow arsenic still strongly affects the ability of these hormone-activated receptors to regulate gene expression. There's still a lot more to learn.”

The study also looked into the effects of different levels of arsenic on these receptors. At very low doses (comparable to what is found in drinking water at the current and previous U.S. regulatory limits, in the range of 5-50 ppb) arsenic enhances hormone-stimulated gene expression, by two- to three-fold. At slightly higher doses (in the range of 50-200 ppb, commonly found in drinking water from contaminated wells in New Hampshire and elsewhere in the U.S.) arsenic has the exact opposite effect, strongly and almost completely inhibiting hormone-stimulated gene expression by these receptors. This non-conventional dose-response suggests that arsenic might have very different biological effects at the lower and higher doses.

“Elucidating these complex biological effects of arsenic on hormone signaling at different doses will be critical to our overall understanding of how arsenic influences human health, and should be considered as an important component of determining the overall disease risk of people who are exposed to arsenic in their drinking water,” says Hamilton.

The work is funded by grants to Dartmouth collaborators Hamilton and Bodwell from the National Institute of Environmental Health Sciences, a component of the National Institutes of Health. Both researchers are members of the NIEHS-funded Superfund Basic Research Program at Dartmouth and Dartmouth's Center for Environmental Health Sciences. Co-authors on the study include Julie A. Gosse, and Athena P. Nomikos, both of Dartmouth and both recipients of training fellowships from Dartmouth's Superfund Basic Research Program.
Gene-environmental factors affecting risk
Texas A&M University SBRP
Project Leaders: C. Naspinski, Y. Tian, R. Finnell, T.J. McDonald, L. Mitchell and K.C. Donnelly

The potential for toxic mixtures in the environment to impact human health is dependent on many factors. While it is apparent that the concentration and composition of the mixture have a significant effect on toxicity, the interactions of genetic and nutritional factors are less evident. Cell culture assays provide a useful tool to investigate the impact of genetic factors on chemical toxicity. Pregnane X receptor (PXR) is a recently characterized general xeno-sensor that has been shown to be important for metabolism of numerous environmental contaminants including PAHs. Researchers from several SBRP-funded projects at TAMU have developed a PXR-based cell culture model to analyze the role of PXR in metabolism of benzo(a)pyrene (BaP). Studies conducted in HepG2 liver cancer cells indicated that the presence of PXR significantly decreased the genotoxicity of BaP.

Additional studies confirmed that the presence of the PXR receptor increased the detoxification and elimination of the carcinogens through increased expression of the Phase II enzyme glutathione transferase (GSTM1). Measurement of DNA adducts in placenta from a birth defects study (Figure 1) indicates that placenta from children with a Neural Tube Defect appear to have higher levels of bulky DNA adducts and reduced ability to eliminate carcinogens. On-going studies will measure polymorphisms in GSTM1 to determine if this increases the risk of Neural Tube Defects in children exposed to polycyclic aromatic hydrocarbons (PAHs).

An arsenic metabolite - monomethylarsonous acid: Role in arsenic carcinogenesis
University of Arizona SBRP
Project Leader: A. Jay Gandolfi

Though chronic arsenic exposure increases the risk for development of cancers of the bladder, lung, and skin, millions of people consume drinking water naturally contaminated with arsenic. While it is known that inorganic arsenic is metabolized to five potentially toxic compounds following ingestion, it is not yet known which of these arsenicals contribute to carcinogenesis in the human body. Identification of carcinogenic arsenicals is key to understanding the mechanism of arsenic carcinogenesis.

Recent research has demonstrated that trivalent, methylated arsenic metabolites are more cytotoxic and genotoxic than inorganic arsenicals. Therefore, it is plausible that the trivalent methylated arsenicals, such as monomethylarsonous acid [MMA(III)] and dimethylarsinous acid [DMA(III)], are the ultimate arsenic carcinogens.
To investigate the carcinogenicity of MMA(III), a non-tumorigenic human urothelial (UROtsa) cell line was exposed to an environmentally relevant dose of 0.05 µM MMA(III) for a total duration of 52 weeks. This dose level is equivalent to that detected in the urine of people consuming drinking water contaminated with 200 ppm arsenic.

This work generated a new transformed cell line, URO-MSC52 that has the following characteristics.

1. Hyperproliferation (rapid, excessive growth and reproduction). Hyperproliferation results from dysregulation of cell growth, which often characterizes malignancy. After 12 weeks of exposure, the UROtsa cells exposed to MMA(III) had a growth rate that was twice as fast as untreated cells.

2. Anchorage-independent growth. Most cells require a surface on which to flatten out and divide; i.e., they are “anchorage-dependent”. The ability to form colonies in soft agar (“anchorage-independent growth”) is characteristic of many cancer cell lines. Anchorage-independent growth was observed after 24 weeks of treatment.

3. Tumorigenicity. After 52 weeks of treatment, UROtsa cells were injected into immunocompromised mice resulting in tumor formation, indicating that malignant transformation had occurred in the chronically-exposed cells.

4. Changes in gene expression. Since tumorigenesis is associated with the loss of cell cycle regulation, increased oncogene expression was expected in a malignantly transformed cell line. Indeed, gene array analysis of URO-MSC52 cells showed three well-characterized oncogenes were strongly up-regulated. Members of pro-mitotic, anti-apoptotic signal transduction pathways were also up-regulated. In addition to dysregulated cell cycle progression, tumorigenesis is associated with metastasis via cell invasion and migration through the extracellular matrix. To add further support that URO-MSC52 cells are tumorigenic, these cells were found to have up-regulated matrix metalloproteinases. In summary, gene array data strongly support the hypothesis that chronic MMA(III) exposure induces gene expression changes consistent with malignant transformation.

5. COX-2 induction. Cyclooxygenase-2 (COX-2) causes hyperplasia in bladder cells and is considered a key biomarker in bladder cancer. UROtsa cells treated (acutely or chronically) with 10-50 nM MMA(III) increased COX-2 expression. Since COX-2 is an important mediator that contributes to carcinogenesis via promotion of cell proliferation, inhibition of cell death, induction of angiogenesis, and facilitation of invasion, and it is highly up-regulated both acutely and chronically in the MMA(III)-transformed cells, it is likely that activation of the MAPK pathway and increased COX-2 expression is a plausible mechanism for MMA(III) bladder carcinogenesis.

The findings from these studies are the first to show MMA(III)-induced malignant transformation of a human cell line at a biologically-relevant concentration. These findings identify MMA(III) as one specific arsenical that is likely to participate in arsenic carcinogenesis in vivo. In addition, the URO-MSC52 cells establish a model that can be used to investigate the effects of long-term, low-level arsenic exposure in a human cell line. Finally, this research confirms that arsenic metabolism in the human body is a process that generates potentially carcinogenic arsenicals, such as MMA(III), that contribute to arsenic carcinogenesis.
Very little evidence exists concerning the possible impairment of children’s intellectual function in relation to arsenic exposure *in utero* and childhood. Children worldwide are exposed to arsenic in drinking water at concentrations that exceed the standard recommended by the World Health Organization and the U.S. Environmental Protection Agency maximum contaminant level of 10 µg/L. Children are particularly at risk for high exposure in arsenic-affected areas of South Asia such as West Bengal, India.

Acute neurotoxic effects of arsenic in high doses have been well documented. Arsenic poisoning related to occupational exposure causes central nervous system alterations, including impairments of recent memory, learning, and concentration. Children may be particularly susceptible to neurotoxic substances as suggested by findings from studies on the effects of lead, methylmercury, solvents, and PCBs. Experimental animal and petri dish studies, and some limited evidence from the few earlier reports considering children’s intellectual function and arsenic, suggested possible associations between arsenic exposure and neurodevelopment. A recent study conducted by SBRP-funded researchers at the University of California, Berkeley included 201 children in Bangladesh and results suggested that arsenic concentrations in water as low as 10 µg/L were linked to reductions in intellectual functioning in 10-year-old children.

The researchers conducted a cross-sectional study among 351 children aged 5 to 15 years selected from a source population of 7,683 people in West Bengal, India, 2001-2003. Intellectual function was assessed with six subtests from the Wechsler Intelligence Scale for Children, as well as with the Total Sentence Recall test, the Colored Progressive Matrices test and a pegboard test. Arsenic in urine and lifetime water sources including the pregnancy period were assessed using measurements of samples from 409 wells. The test scores were analyzed with linear regressions based on the method of generalized estimating equations incorporating relevant covariates.

This study systematically addresses arsenic exposure from all water sources used over a lifetime (including the pregnancy period), as well as urinary arsenic concentrations, in relation to intellectual function in children. Effects were found for the vocabulary, picture completion, and object assembly tests with reductions between 12% and 20%, but the confidence intervals were broad. The findings suggest that increased urinary arsenic concentrations reflecting current exposure from all sources, including food, are associated with small decrements in intellectual function testing, whereas little evidence for an effect of long-term arsenic concentrations in drinking water was found.

These findings suggest that arsenic exposure measured in urine is related to decrements in intellectual function scores, which may be in the range of 10% to 20% for some tests. However, the 95% confidence intervals of these estimates were wide. Whether or not these effects have persisting impact needs further investigation. There was little evidence of an association between arsenic drinking water concentrations alone and intellectual function. Current urine concentrations reflecting exposure from all sources appeared to be more relevant than pregnancy, peak, or cumulative exposure based on measurements of water sources. One possible explanation is that the relationship with current exposure relates only to transient effects. However, it is also possible that the lack of findings with past water concentrations is due to incomplete assessment of past exposure, in particular, exposure originating from food. Although the findings need to be confirmed, they add to the body of evidence of adverse health effects in children resulting from exposure to arsenic.
**Development and applications of integrated In vitro and cell-based bioassays**
University of California, Davis SBRP
Project Leaders: Michael Denison and Bruce Hammock

This research improves our understanding of signal transduction pathways and their influence on disease. This past year investigators have determined how a group of enzymes, the epoxide hydrolases (EHs), interact with the arachidonic acid (AA) cascade and thereby affect inflammation. Additionally they have found that soluble epoxide hydrolase inhibitors (SEHi) dramatically improved an organism’s response to bacterial endotoxins that typically elicit an inflammatory response, by down-regulating inflammation and acting to reduce pain and by helping to maintain blood pressure at normal levels and have also found therapeutic benefits of combining nonsteroidal anti-inflammatory drugs (NSAIDs) and sEH, i.e., in reducing pain and inflammation, while decreasing the side effects of NSAIDs, e.g., CELEBREX®

**Susceptibility to liver damage correlation with production of the cytokine interleukin 6**
University of California, San Diego SBRP
Project Leaders: Michael Karin and Hyam Leffert

Drs. Michael Karin and Hyam Leffert have been studying how two Superfund chemicals, diethylnitrosamine (DEN) and carbon tetrachloride (CCl4), elicit liver toxicity. They found that male mice were far more susceptible to induction of liver damage following exposure to either DEN or CCl4 than female mice. Susceptibility to liver damage correlated with production of the cytokine interleukin 6 (IL-6), whose expression is induced in response to DEN or CCl4 exposure. The UC-San Diego research team found that quite a bit more IL-6 is produced in male mice than in female mice after toxin exposure. Using IL-6-deficient mice we found that IL-6 plays an important role in amplification of the initial necrotic injury caused by either DEN or CCl4 exposure. Thus, male mice produce more IL-6 and also exhibit more liver injury than females after DEN or CCl4 exposure. Importantly, the absence of IL-6 reduced toxin-induced liver injury by 70-80% and also reduced compensatory proliferation and hepatocarcinogenesis by more than 90%.

IL-6 is produced by Kupffer cells (liver macrophages) and the researchers found that its expression by Kupffer cells in males is strongly inhibited by estrogen. Administration of estrogen to male mice reduced DEN- and CCl4-induced liver injury. These results explain gender disparity in susceptibility to liver damage and suggest that estrogens can be used to reduce liver damage in individuals exposed to liver toxins found at Superfund sites. Furthermore, inhibition of IL-6 production can be used to prevent the progression of various liver diseases associated with liver damage and inflammation to hepatocellular carcinoma. These exciting findings may lead to novel clinical studies aimed at predicting predisposition to environmentally induced liver disease.
Estrogen receptor activation by xenogenic estrogens
University of California, San Diego SBRP
Project Leaders: Ron Evans

The nuclear receptor (NR) pregnane X receptor (PXR) acts as a major xenobiotic sensor that protects the body from a multitude of environmental toxicants and plays a central role in the metabolism and clearance of steroids and toxic endogenous lipids. To understand the nature of the recognition diversity and similarity of human and mouse PXR isoforms Dr. Ron Evans’ group has screened several commercially available chemical libraries (Microsource Discovery Systems Inc) for human PXR activators using cell based luciferase reporter assays. Initial and secondary screening was carried out in duplicate using rifampicin and PCN used as human specific and rodent specific control ligands, respectively. The screening produced 43 hits against the human PXR and 32 hits against the mouse PXR.

A parallel project explored the observation that the human estrogen receptor is known to be activated by a number of environmental naturally occurring plant compounds. In addition certain environmental polycyclic aromatic hydrocarbons are also ER activators. These compounds, collectively referred to as xenogenic estrogens, have been proposed to contribute to endocrine disruption syndromes in people. While the basis for endocrine disruption is not clear, the estrogen receptor directly binds 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. To test this hypothesis, the Evans group first screened several commercially available chemical libraries for ER\(\alpha\) and ER\(\beta\) activators using high-throughput cell-based luciferase reporter assays. The libraries that they tested initially included Microsource Gen-Plus (960 compounds), new and classical therapeutic agents and the Microsource Pure Nature Products (720 compounds).

They performed the initial and secondary screens in triplicate with estradiol (E2) as a control. Surprisingly, 184 out of these 1680 compounds activated ER\(\alpha\) with a similar or higher luciferase activity than estradiol, and 35 out of 1680 compounds could activate ER\(\alpha\). The categories of chemicals that could activate either ER\(\alpha\) or ER\(\beta\) include a diversity of chemical structures. Some of the compounds that activated both ER\(\alpha/\beta\) 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. This study suggests a new molecular mechanism by which environmental substances may contribute to adverse medical events, through the unanticipated activation of ER and induction of hepatic drug metabolism.

The impact of obesity on PCB toxicity
University of Kentucky SBRP
Project Leader: Lisa Cassis

The Commonwealth of Kentucky has numerous hazardous waste sites on the national priority list. Thus, Kentuckians are at risk of exposure to Superfund chemicals, including polychlorinated biphenyls (PCBs). Obesity is at epidemic proportions in the US, with 64.5% of the adult US population considered overweight. It is well known that obesity predisposes to many different diseases, with cardiovascular disease accounting for the greatest mortality in obese individuals. Kentucky consistently ranks above the national average in the prevalence of overweight and obesity, and is 4th in the nation in deaths from cardiovascular disease.
Because of their chemical structure, PCBs accumulate in the lipid pools of fat cells, or adipocytes. Adipocytes are the main cell in fat tissue within the body. Adipocytes store lipid for energy needs in the body, and release lipid in response to specific stimuli. Adipocytes also produce a wide variety of substances, including several factors that stimulate inflammation. With obesity, the lipid pools of adipocytes increase markedly, and this further stimulates inflammation within fat tissue. Despite the marked accumulation of PCBs in adipose tissue, little is known as to how or whether they influence fat cell function. Importantly, adipocytes do not divide and proliferate; thus, to form a new fat cell a precursor cell must be stimulated to differentiate or transform into an adipocyte. Dr. Lisa Cassis used a model fat cell culture system to examine the effects of PCBs on the recruitment of new fat cells, and on the inflammatory status of formed fat cells. Her results demonstrate that exposure of the precursor cell to PCBs results in conversion of these cells to mature adipocytes that have an increased amount of lipid. This result would suggest that exposure to PCBs would promote or increase the development of obesity. Dr. Cassis’ group also examined the expression of various genes, focusing on substances that promote inflammation in adipocytes. Their results demonstrate that exposure of the adipocyte to PCBs increases the gene expression and release of a wide variety of pro-inflammatory factors. These results suggest that exposure to PCBs may not only increase obesity, but could also promote the inflammation in adipose tissue that links obesity to cardiovascular disease.

To determine if these effects obtained in cell culture are relevant to what happens in the body, the researchers treated mice with PCBs and examined body weight gain, atherosclerosis (coronary artery disease), and abdominal aortic aneurysm (AAA) formation. They used a mouse model that they previously created. When certain types of mice are infused with the peptide angiotensin II (AngII), the mice exhibit the vascular diseases of atherosclerosis and profound AAAs. The researchers treated the mice twice with PCBs and examined AngII-induced vascular diseases. They noted that mice treated with PCBs exhibited an increase in their body weight. This result supported our findings described above in the adipocyte cell culture system, suggesting a role for PCBs in obesity. They also noted a very pronounced effect of PCB treatment to cause lipid or fat deposition abnormally within tissues, most especially skeletal muscle. Importantly, mice treated with PCBs exhibited very severe AAAs, to the point that several of the mice died from ruptured aneurysms. Rupture of aneurysms in humans is the leading cause of death from this disease. In addition, atherosclerosis was increased in mice treated with PCBs. Dr. Cassis’ group is currently examining the role that adipose or fat tissue plays in these effects of PCBs. Interestingly, adipose tissue surrounds major blood vessels, including the aorta, the site for AAA formation. Their hypothesis is that PCBs promote inflammation in the fat tissue surrounding the aorta, and thereby increase vascular disease. In addition, the development of obesity from PCB exposure would be anticipated to worsen cardiovascular disease.

The public health impact of these studies is that exposure to PCBs may contribute to or worsen the epidemic of obesity in the US, as well as promote cardiovascular diseases associated with obesity.
The pollution of natural waters from industrial and agricultural waste has resulted in widespread chemical contamination that is in need of remediation. Natural processes that degrade pollutants include sunlight photolysis and oxidation, as well as microbial activity. Engineered systems can accelerate the destruction of chemical contaminants through advanced treatment pathways similar to these natural processes. Both polycyclic aromatic hydrocarbons (PAHs) and organophosphate pesticides (OPs) are classes of chemicals that are of concern for both human health and natural ecosystems. Some of these chemicals degrade very slowly, so technologies to increase their degradation rates are of interest.

In addition, it is not well known whether degradation of parent compounds necessarily decreases toxicity. The fate of these chemicals during natural environmental decay, effects of degradation on toxicity, and development of strategies for remediation using ultraviolet light based photolysis and oxidation and enhanced microbial degradation are under study in the laboratories of Drs. Schuler and Linden.

Microbial studies have focused on the degradation of PAHs and microbial population dynamics in mixed bacterial cultures established by enrichment from creosote-contaminated sediments from a salt-marsh tidal inlet along the Elizabeth River adjacent to the Atlantic Woods Industries Superfund site in Virginia. The researchers are investigating degradation of the PAH dibenzothiophene (DBT) with and without pH control, while monitoring degradation, microbial population dynamics, degradation products, and changes in toxicity. Microbial population dynamics of 10 taxonomic groups were examined using quantitative PCR (qPCR) assays, and toxicity was measured as luminescence inhibition in the bioluminescent bacterium *Vibrio fischeri*. Without pH control, pH dropped substantially and DBT degradation was greatly reduced. When pH was maintained at 7.5, however, degradation proceeded steadily and over 90% reduction was achieved.

Based on the bioluminescence assay, it appears that the formation of toxic intermediate products is an important concern, as toxicity initially increased and stayed at relatively high levels even after the parent compound was degraded, suggesting that removal of the parent compound alone may be a poor indicator of bioremediation. According to qPCR results, Flavobacteriaceae and Chromatiales- groups were dominant regardless of pH control, while Rhizobiales-like bacteria were a major group under no pH control. None of these organisms have been previously implicated in DBT degradation, but Chromatiales is known to accumulate sulfur and so may be of particular interest in the degradation of this sulfur-containing PAH. These studies are some of the first using qPCR to monitor microbial populations in a mixed culture during degradation, and the results suggest that the combined contributions of the microbial consortium are important to understanding and improving biodegradation of even simple PAH containing systems.

Fundamental parameters of UV-based oxidative processes (UV and UV/H2O2), including degradation kinetics for the polycyclic aromatic hydrocarbons (PAHs) fluorene (FLU), dibenzofuran (DBF) and dibenzothiophene (DBT) alone and in mixtures were developed and published to support the design of UV-based remediation systems. A synergistic effect was observed during direct photolysis of the mixture as compared to photodegradation as a single component in an aqueous solution. A simulated natural sunlight system was constructed to study natural PAHs photolysis processes. Experiments showed no direct photolysis of FLU, DBF, or DBT during 8 hours of exposures.
The kinetics and mechanisms of the photodegradation of the organophosphate pesticides chlorpyrifos, parathion, and diazinon were also investigated under engineered and natural UV decay conditions. Fundamental parameters of UV-based decay were developed to allow modeling and design of a treatment system for destruction of these chemicals. In natural systems, the sunlight itself did not result in appreciable decay of the contaminants, but the enhancement due to photolysis in the presence of naturally occurring photosensitizers is currently under study.

The relationship between reduction of parent compound and reduction of actual toxicity is being made with the assistance of the Freedman Lab using the nematode C. elegans to evaluate changes in lethality, reproduction, food consumption and feeding behavior. These studies have been complemented through collaboration with Professor Slotkin investigating the extent of specific acetylcholinesterase inhibition post-UV treatment of the OPs utilizing neural cells (PC12) as the model system. The bioluminescence inhibition bioassay was also applied to the products of the photolysis/oxidation experiments, and toxicity was correlated with a reduction in concentration of the parent PAHs, including DBT (as sole component and in a mixture), which is contrasted with the microbial degradation results. Future work will assess the combined photolytic/oxidation and microbial degradation effects on degradation kinetics of DBT and associated toxicity reduction.

**Molecular insight into polyaromatic toxicant degradation by microbial communities**

Michigan State University SBRP

Project Leader: James M. Tiedje, Ph.D.

The major goal of Dr. Tiedje’s SBRP-funded project is to better understand the microbial degradation of halogenated polyaromatic compounds, mainly those with dioxin-like activity (polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dioxins (PCDDs), under both aerobic and anaerobic conditions. This is important because microorganisms play a major role in the environmental fate of these compounds. A portion of this work involves investigating the regulation and control of biodegradative pathways in Burkholderia xenovorans LB400, the most effective aerobic PCB degrading microorganism known. A second portion involves discovering new microbial capabilities for the degradation of halogenated aromatics. Dr. Tiedje’s group suspects that existing information regarding such capabilities is quite limited because it has come primarily from traditional culture-based studies, and more than 99% of microbial species have yet to be cultured. Accordingly, they are using a variety of molecular, culture-independent, methods to explore and recover genes for two key classes of enzymes involved in the transformation and detoxification of chlorinated polyaromatic compounds from the DNA of this uncultured microbial diversity. These key enzymes are, for aerobic environments, the dioxygenases, and, for anaerobic environments, the reductive dehalogenases. The genes they discover can then be used as markers of specific metabolic capabilities. Dr. Tiedje intends to use them as such to aid in site assessment and to make qualitative and quantitative predictions of the biodegradation that can be expected at contaminated sites. The information they gain may also ultimately be used to develop advanced bioremediation strategies.

**Regulation of Degradative Pathways**

Burkholderia xenovorans LB400 is arguably the best aerobic PCB degrader known. Not only is it capable of degrading a greater variety of PCB congeners than most (due to characteristics of its dioxygenases), but it is less inhibited by the accumulation of intermediate PCB metabolites than other species. This is undoubtedly related to several novel characteristics Dr. Tiedje’s group has discovered through genomic and proteomic studies on various carbon sources in the presence and
absence of PCBs. These characteristics include: i) a redundancy of the lower branch of (PCB)biphenyl metabolism implicating a complex co-regulation of at least three major pathways for monoaromatic ring metabolism, ii) involvement of active transport mechanisms in biphenyl metabolism, and iii) a global switch-on of a C1 metabolic circuit during transition to stationary phase in biphenyl grown cells. These data suggest either a developmental phase-dependent or scavenging (low nutrient) controlled induction that could be critical to in situ PCB degrading capabilities.

Dr. Tiedje's group has outlined the potential regulators and transporters associated with the functioning of the (chloro)biphenyl metabolic network, and is now constructing knockout mutations of the potential regulators and transporters to determine their significance to PCB degradation. Completion of this work will reveal why LB400 is an exceptional PCB degrading microorganism.

**Aerobic Dioxygenases**

Dr. Tiedje's group has begun aerobic work by enriching for dibenzofuran degrading microorganisms in sulfidogenic sediments from the Passaic River, NJ. Community profiling revealed a decrease in species richness and strong selection for a Paenibacillus naphthalenovorans-like phylotype. At the same time they observed an increase in the diversity of Rieske-type oxygenase genes. The data suggest that historically PCDD and PCDF contaminated anoxic sediments can harbor a wide range of degrading bacteria allowing for a succession of dominant phylotypes during different stages of pollutant degradation.

The researchers have also applied stable isotope probing (SIP) in aerobic experiments aimed at recovering genes and operons involved in the degradation of (chloro)biphenyls. The advantage of SIP is that it provides a way of enriching a sample for DNA from the degrading microorganism(s). 13C-biphenyl was fed to biphenyl degrading River Raisin (Monroe, MI) sediment slurries. After allowing time for the 13C to be incorporated into DNA, the DNA was extracted from the sediment. The heavy fraction of DNA (from microorganisms that metabolized the biphenyl) was separated by centrifugation in a CsCl gradient and used to construct a cosmid library of 1,700 clones. PCR-based screening of this library using universal biphenyl oxygenase primers identified several candidate clones which we are now sequencing. Operons so obtained will be tested for their involvement in PCB degradation and for the congener specificity of their dioxygenases.

**Anaerobic Dehalogenases**

Dr. Tiedje's group has begun anaerobic work using PCB dechlorinating microcosms originally inoculated from Hudson River sediments. These have been transferred several times under various conditions including pasteurization of the inoculum between transfers, and transfer to PCB-free microcosms. Three known dehalogenating genera (Dehalococcoides, Dehalobacter, and Desulfitobacterium) have been present in all non-pasteurized treatments with PCBs, and the first two increase in abundance during periods of maximum dechlorination activity. Also, Dehalococcoides was lost after transfer to PCB-free microcosms. Thus Dehalococcoides and Dehalobacter are most likely responsible for PCB dechlorination. Pasteurization resulted in the loss of Dehalococcoides (but not Dehalobacter or Desulfitobacterium) from the microcosms and a recognizable change in the dechlorination pattern. Pasteurized treatments removed chlorines only from meta positions, while non-pasteurized treatments also removed para chlorines from certain PCB congeners. The researchers have obtained 24 Dehalococcoides-like putative dehalogenase sequences from the non-pasteurized treatments and two more, similar to dehalogenase sequences from Dehalobacter and Desulfitobacterium, from the pasteurized treatments. Thus they can begin making tentative associations between dehalogenases and the congener specificity of PCB dechlorination.

This research will ultimately cover the degradation of PCBs, PCDFs, and PCDDs under both aerobic and anaerobic conditions. To verify the role of genes they discover, Dr. Tiedje's group will construct metagenomic libraries and probe them for
the genes of interest. Selected clones will be expressed in appropriate heterologous hosts and tested for their abilities to
effect transformation of the target contaminants. The information generated will be integrated into quantitative diagnostic
tools based on Bayesian probabilistic networks to predict the potential, rate, and extent of biodegradation at a contaminated
site by mathematically integrating information from genomics, physiology, and geochemistry.

Dr. Tiedje expects this research to provide significant contributions to basic science. The angular dioxygenases and reductive
dehalogenases are relatively newly discovered classes of microbial enzymes about which little is known, and this work will
provide new information on these enzymes and other aspects of microbial catalytic diversity that is not easily obtainable by
traditional (culture dependent) methods.

The major practical outcome of the project is that it will lead to better predictions of the behavior of contaminants in particu-
lar types of environments, thus decreasing the uncertainty in risk and exposure assessments. The knowledge gained may be
used to improve the design and control of bioremediation processes and provide tools for better monitoring of intrinsic pro-
cesses, leading to decreased human exposure to toxicants. And while Dr. Tiedje’s group is working with soils and sediments
from certain federally and state listed hazardous waste sites, the results should be translatable to any contaminated site.

Remediation of metal mixtures
Michigan State University SBRP
Project Leader: Craig S. Criddle, Stanford University

Dr. Criddle’s earlier SBRP-funded work focused on elucidating the structure of menaquinone-1 and its ability to reduce ferric
iron and carbon tetrachloride. His findings show that menaquione-1 is capable of both transforming carbon tetrachloride
as well as reducing iron (III) species.

In 2006, Dr. Criddle’s group focused on the secreted factor Pyridine-2,6-bis-thiocarboxylate (PDTC) from Pseudomonas
stutzeri KC for its transformation on carbon tetrachloride. PDTC secreted from Pseudomonas stutzeri KC possesses a unique
mechanism of transforming carbon tetrachloride into carbon dioxide. Production of PDTC from Pseudomonas stutzeri KC
was measured in the presence and absence of carbon tetrachloride by liquid chromatograph tandem mass spectrometry (LC/
MS/MS). Production of PDTC coincided with the onset of carbon tetrachloride transformation. Additional carbon tetra-
cloride spikes were added at concentrations. These were quickly transformed. This confirms earlier work establishing that
the carbon tetrachloride transformation is catalytic and that PDTC-Cu was regenerated for transformation at a rate equal to
or greater than the rate of carbon tetrachloride transformation. The results indicated that the presence of carbon tetrachloride
allows rapid regeneration of PDTC and enhances the transformation capacity for carbon tetrachloride tranformation. The
enhancement of this activity is evident by the large amount of carbon tetrachloride transformed by apparently small amount
of PDTC measured during the active transformation. A manuscript is in preparation (Regeneration of PDTC activity allows
long-term carbon tetrachloride degradation by Pseudomonas stutzeri KC, (Fu et al., in preparation)

Work also focused on clarifying the mechanism of TCE and cis-DCE degradation by synthetic nano-scale mackinawite.
Synthetic mackinawite is capable of reductive dechlorination of TCE and cis-DCE, with the TCE degradation rate orders
of magnitude faster. Often considered non-reactive with cis-DCE, freshly precipitated nano-scale mackinawite particles
were found capable of reductive degradation of cis-DCE into acetylene. From these studies, the researchers have identified
several factors that enhance the reactivity of chemically synthesized mackinawite particles. First, the finer fraction of syn-
thetic mackinawite produced during its synthesis, which is nano-scale in size, is the most important fraction in determining FeS reactivity with TCE or cis-DCE. As such, care should be taken not to wash out this fraction. When conventional sample preparation routines involving rinsing and free-drying are used, this fraction is often washed away is one reason why previous studies have found little or no reactivity of FeS for cis-DCE. Second, the synthesis pH of FeS preparation can be an important parameter in the ultimate reactivity of the synthetic iron sulfide particles. Results indicate that the higher the pH of synthesis (from 7 up to pH 10), the more reactive are the particles. Third, the iron:sulfur ratio used in during the mackinawite synthesis strongly affects the reactivity of the FeS solid formed. We found that a ratio close to 1:1 was the most favorable condition for producing highly reactive particles. Finally, adding citrate during the iron sulfide synthesis improved the synthetic mackinawite’s reactivity as well as its stability under oxidizing conditions. In the absence of citrate, when synthetic FeS is exposed to air, rapid FeS oxidation occurs within hours with the formation of an brown solid but in the presence of citrate the FeS resisted oxidation with the solid maintaining its black color. These results have important implications for preparing FeS for permeable reactive barrier applications indicating that optimal reactivity of FeS requires retention of fines, pH control, and Fe:S ratio during synthesis and the desirability of using agents such as citrate to protect against unwanted air oxidation during synthesis, storage and handling.

**Arsenic in water: Removal technologies and residuals disposal**

*University of Arizona SBRP*

*Project Leader: Wendell Ela*

An unprecedented mass of arsenic is being sent to non-hazardous landfills as the disposal of arsenic treated lumber from construction coincides with the increasing disposal of arsenic-bearing solid residuals (ABSR) from water treatment. Past research shows that arsenic is readily mobilized under landfills conditions, particularly from ABSR, which are estimated to have an arsenic release potential 100 times greater than that of treated timber (i.e., 1 pound of arsenic in ABSR will cause the same arsenic impact on landfill leachate as 100 pounds of arsenic in treated timber).

This project focuses on means to mitigate the likelihood that ABSR disposal will create an environmental contamination legacy. Three research thrusts dominated the 2006 year’s work:

1) Development of a selective, regenerable arsenic sorbent as an alternative to the current throw-away sorbents. A new type of arsenic sorbent is undergoing initial testing. The polymeric sorbent is functionalized by incorporating metal ions with strong, specific affinity for arsenic (e.g., iron and copper). Early tests of the polymers show high arsenic capacity, which is not diminished by the presence of other common water constituents such as sulfate, silica and phosphate. The sorbents can, in principle, be regenerated indefinitely simply by pH manipulation.

2) Understanding the mechanisms causing arsenic release from ABSR under landfill conditions. Conditions of interest include:

- Aging of ABSR. In order to better understand and control the mechanisms of ABSR arsenic release, trials were conducted to assess the impact of ABSR aging on the release of sorbed arsenic. The work indicates an aging process that binds the arsenic more strongly over time, although in parallel work the overall capacity of certain sorbents may also simultaneously decrease. Quantum mechanical simulations were performed to determine the effect of arsenic adsorption to iron oxides on the equilibrium redox potential
between arsenate, the arsenic species in ABSR, and arsenite, the more mobile species generated under landfill conditions. These simulations indicate that the stronger binding of arsenate versus arsenite onto ABSR decreases the equilibrium potential and makes arsenate reduction less favorable. Thus, strongly bound arsenate is thermodynamically less favored than desorbed arsenate for reduction to the more toxic and mobile arsenite form.

• **Microbial Effects.** Previous work on the effect of microbial activity on arsenic release from alumina ABSR was expanded to include studies of release from iron-based (the currently most common sorbent type) and titanium-based (the next generation sorbent emerging from industry) commercial sorbents. Under simplistic simulated landfill conditions, microbially active samples of both sorbents leached 4-5 times greater arsenic than their abiotic analogs and greater than 20% of the total arsenic was released in less than 9 months. However, the longer-term leaching rate from titanium was less than iron media.

Based on these findings, Dr. Ela’s group can predict that segregated disposal of ABSR into landfill cells lacking organic matter (and therefore minimizing microbial activity) is a simple, inexpensive means to greatly reduce arsenic release.

3) **Stabilization of ABSR to mitigate their arsenic release rate.** Work in 2006 has also shown the promise of two types of ABSR treatment, encapsulation and crystallization, to mitigate ABSR leaching.

• **Encapsulation.** A polymeric encapsulation technology has been developed through the proof-of-concept phase and shown to successfully incorporate greater than five-fold greater ABSR loading than comparable cement treatments, while decreasing leaching under standard test conditions by 100 to 1000 fold compared to untreated and cement treated ABSR. Preliminary results of the encapsulated ABSR under simulated, fully biotic, landfill conditions show arsenic release decreased by over 100 fold.

• **Crystallization.** The crystallization technology aims to transform the arsenic into natural, leach resistant mineral phases through low temperature and pressure processes. Scorodite, apatite and siderate, all natural arsenic-bearing minerals, were successfully formed in the laboratory at temperatures less than 160°C. Both scorodite and siderite forms show sub-parts-per-million arsenic solubility, despite containing greater than 20% by weight of arsenic.

The scientific results of this project were the motivation for two national workshops in 2006. At these workshops, the implications of new findings on the relationship between arsenic and landfills with particular emphasis on current management strategies for disposal of arsenic solid wastes and landfill mediated releases of arsenic from natural and manmade solids was discussed. Federal, state, local and private stakeholders attended the workshops and the discussion of implications on waste-site clean-up, landfill operation and solid-waste disposal is actively on going.
This project seeks to understand and optimize the microbial detoxification of common Superfund pollutants, perchloroethylene (PCE) and trichloroethylene (TCE) by focusing on the only genus of bacteria, Dehalococcoides, known to completely reduce PCE and TCE to ethene. A better understanding of the genome, transcriptome and proteome of Dehalococcoides will greatly improve our understanding of the physiology of these difficult to grow organisms, so that their abundance and activity at bioremediation sites can be maximized.

Drs Alvarez-Cohen and Anderson determined that Dehalococcoides strain BAV1 diverges from that of D. ethenogenes 195 in several major metabolic pathways. Conversely, we determined that ANA enrichment possesses most of the same genes as D. ethenogenes 195 suggesting significant horizontal gene transfer among species.

The research groups measured the transcriptomic effects of cobalamin (vitamin B12)-limited growth conditions. This stress condition is of particular relevance because cobalamin is a necessary co-factor for reductive dehalogenases but cannot be biosynthesized de novo by this isolate. The findings revealed a cobalamin regulon in this isolate and provided novel genetic targets for monitoring cobalamin stress in Dehalococcoides spp.

Chlorinated solvents are the most common groundwater contaminants at Superfund sites. In situ bioremediation is a promising and cost effective method for remediation of these contaminants. The aim of project 4 is to improve prediction and monitoring which will ultimately optimize system performance at remediation sites.

One aspect of the work involved an enriched anaerobic microbial community (ANAS) that reductively dechlorinates chlorinated ethenes including TCE, cis-dichloroethene (c-DCE) and vinyl chloride (VC). The researchers used whole genome microarrays based upon the chromosome of D. ethenogenes 195 to compare the genomics of strain 195 to those of Dehalococcoides strain BAV1 and the ANAS enrichment. Thus far, they have determined that the genome of strain BAV1 diverges from that of strain 195 in several major metabolic pathway genes including genes typically involved in carbon assimilation, nitrogen fixation, cobalamin usage, hydrogenases, and especially reductive dehalogenases. Conversely, the ANAS enrichment possesses most of the same genes found in strain 195, with the exception of a number of viral inserts containing reductive dehalogenase genes, suggesting significant horizontal gene transfer among species.

In addition, they applied whole-genome microarrays to compare the transcriptome of strain 195 when grown under differing growth conditions. In particular, the researchers measured the transcriptomic effects of cobalamin (vitamin B12)-limited growth conditions. This stress condition is of particular relevance because cobalamin is a necessary co-factor for reductive dehalogenases but cannot be biosynthesized de novo by this isolate. The findings revealed a cobalamin regulon in this isolate and provided novel genetic targets for monitoring cobalamin stress in Dehalococcoides spp.

In a related study, they characterized the effects of exposing strain 195 to cell-free supernatants obtained from the ANAS enrichment. To the group’s surprise, the genes that were most strongly down regulated after exposure to the supernatants were involved with cobalamin salvage and recycling, suggesting that members of ANAS biosynthesize additional cobalamin de novo that can be transferred to Dehalococcoides. The researchers intend to further characterize this eco-physiological interaction as it may be crucial for avoiding cobalamin stress and prolonging dechlorination activity in the environment.

Progress also includes the assembly of defined consortia from combinations of Dehalococcoides and other key species iden-
tified in the ANAS enrichment. The researchers were able to grow Desulfovibrio vulgaris Hildenborough (DVH) with strain 195 in syntrophy on a defined medium containing no hydrogen and no sulfate. They will apply qPCR and whole-genome microarrays to compare the transcriptomes of DVH, 195, and the co-culture DVH/195 and to identify genes important in these symbiotic interactions.

The research group has completed the molecular analysis of a microbial community through different phases of enhanced bioremediation at Ft. Lewis East Gate Disposal Yard (Seattle, Washington), a TCE contaminated field site undergoing in situ bioremediation. They applied qPCR to measure the 16S rRNA gene and the three functionally important reductive dehalogenase genes (tceA, bvcA, vcrA) of Dehalococcoides spp. present in the groundwater through different stages of treatment process (biostimulation and bioaugmentation). Quantification of gene concentration and expression suggests that Dehalococcoides spp. were not only present but were physiologically active in the field communities and provided a clear trend of the dynamics of the different strains of Dehalococcoides in response to the manipulations at the site.

Finally, the group expanded their analyses to include application of a whole genome microarray to measure the expression of genes by Rhodococcus sp. RHA1 in response to induction by propane. In these studies they have shown that the propane monooxygenase is inducible and responsible for NDMA degradation by this organism.
Atmospheric sources of PCB congeners
University of Iowa SBRP
Project Leaders: Keri Hornbuckle and Dean Macken

Airborne PCBs are being measured throughout urban Chicago. University of Iowa SBRP investigators have designed and installed an innovative high volume (Hi-Vol) sampling system. Keri Hornbuckle, associate professor of environmental engineering, and Dean Macken, director of the Iowa Engineering Design and Prototyping Center collaborated on design, construction and installation of two Mobile Hi-Vols. The two systems are now installed on two health clinic vans that serve children with asthma and their family members.

The investigators are studying sources of airborne PCBs in urban and industrial areas of Chicago and Northwest Indiana. This region experiences high concentrations of airborne PCBs because PCBs were commonly used in heavy industries active in the region between 1930 and 1970. PCBs continue to contaminate air in cities like Chicago because they volatilize into the air from old spills and other disposal sites. But such spills are hard to find. Measuring regions of high air PCB concentrations – hot spots – would be a good way to find the sources, but deployment of multiple samplers is prohibitively expensive and logistically difficult.

The high volume air sampler (Hi-Vol) equipped with XAD resin and quartz fiber filter is the most widely accepted method for collecting gas and particle-phase PCBs. But Hi-Vols are expensive, require an electrical outlet, and must be serviced daily. Hi-Vols have limited utility in cities because of the inconvenience and cost of deploying, maintaining, servicing, and protecting them. The Mobile Hi-Vols designed at the University of Iowa are an innovative solution.

Figure 1. Rear view of Mobile C.A.R.E. van with Hi-Vol deployed. During transit, the filter, XAD, and pump assembly is lowered and stored in the locked trunk.
The University of Iowa investigators have constructed a framework for holding a Hi-Vol on the rear of a medical clinic vehicle. The Hi-Vol itself was purchased from Tisch Environmental, Inc (Cleves, OH). The Hi-Vol includes a vacuum motor that pulls air through a quartz fiber filter. The Hi-Vol was modified at the University of Iowa to hold XAD resin in an aluminum screen cartridge. The XAD cartridge holder was installed between the quartz filter and the vacuum motor. With this design, airborne particles are captured on the filter and gases are captured on the XAD resin. The entire modified Hi-Vol has been mounted on an aluminum frame. This frame is attached to a trailer hitch and to a three meter long screw on one of two health clinic vans. The frame and screw allows the Hi-Vol to be elevated into a high position for sampling. When in the down position, the Hi-Vol is stored in a sealed compartment. The Hi-Vol is operated in the up position over the roof of the vehicle. Using this system, the Hi-Vol and samples are protected when the vehicle is in transit and samples are collected in the open air when the vehicle is on site. A sensor that records temperature and relative humidity is also mounted on the rear of the vehicle. This data is needed because gas-phase PCB concentrations often show a relationship with air temperature.

The mobile Hi-Vols are operated with the assistance of the staff at Mobile C.A.R.E. Foundation of Chicago. The Mobile C.A.R.E. Foundation is a non-profit organization dedicated to providing free asthma care and education to children in underserved areas of Chicago. The mobile Hi-Vol system is now operating on Asthma Vans I and II. These two vans serve forty schools in Chicago and operate 12 months of the year. The vans’ schedule require them to park at a school for a day, or about 6-8 hours. Upon arrival at the school, the van driver opens the protective trunk and switches on the elevator screw which raises the Hi-Vol into position over the roof of the van. The drive then switches on the Hi-Vol itself. At the end of the day, the driver turns off the Hi-Vol and turns on the motor that moves the sampler into the down position in the protective trunk. The trunk is closed and the van returns to the garage where Mobile C.A.R.E. staff unload the day’s samples and reload clean filters and XAD for the next day.

The mobile samplers will collect 2 samples every day that the Asthma Vans are operating. Hornbuckle anticipates that over 300 filters and 300 XAD samples will be collected each year. Blanks and other quality control samples raise the total expected number of mobile samples to almost 1000 for remaining three years of the isbrp grant.

The samples will be analyzed for 209 PCB congeners using high pressure solvent extraction and high resolution gas chromatography with tandem mass spectrometry. The samples will be analyzed for PCBs by the isbrp Analytical Core, also lead by Dr. Hornbuckle with Co-Leader Dr. Craig Just and Dr. Hans Lehmler.

The results of the congener-specific analyses will be used to identify PCB ‘hot spots’ in Chicago. Once these regions of high concentrations are found, the investigators will using the measurements to calculate emission rates and make recommendations about remediation.
**Development of new exposure assessment technologies**  
**University of California, Davis**  
**Project Leaders:** Ian Kenney, Michael Denison and Bruce Hammock

This research which involves the development of new exposure technologies, promotes interdisciplinary, integrative research approaches, improves and expands access of researchers to advanced technology and scientific infrastructure. The collaborating researchers have demonstrated luminescent, magnetic nanoparticle immunoassays having high sensitivity in miniaturized immunoassay systems with new, optically efficient luminescent labels for biomolecules. Nanoparticles made of lanthanide oxides (e.g., europium, terbium) have unique spectral properties including large Stokes shift (i.e., low overlap between emission and absorption spectra), sharp emission spectra, long lifetime and good photo-stability thereby greatly reducing background signal-to-noise, vastly improving detection limits, increasing sample stability, reducing sample volume requirements, facilitating quantitation and allowing for new inexpensive multiplexed assay formats. Initially developed for environmental applications, the general utility as an analytical detection method for other fields, including clinical medicine is evident. A provisional patent has been obtained for the magneto-microchannel immunoassay.

**Development and Application of Biomarkers of Exposure**  
**University of North Carolina at Chapel Hill SBRP**  
**Project Leader:** S.M. Rappaport

Benzene is a common contaminant at hazardous waste sites and is also emitted from gasoline and in organic combustion products. Although the toxicity of benzene has been linked to its metabolism, the dose-related production of metabolites is not well understood in humans, particularly at low levels of exposure. Dr. Rappaport used natural spline (NS) models to investigate nonlinear relationships between levels of benzene metabolites [E,E-muconic acid (MA), S-phenylmercapturic acid (SPMA), phenol (PH), hydroquinone (HQ), and catechol (CA)] and benzene exposure among 386 exposed and control workers in Tianjin, China. After adjusting for background levels (estimated from the 60 control subjects with the lowest benzene exposures), expected mean trends of all metabolite levels increased with benzene air concentrations over the range from 0.03 to 88.9 ppm. Molar fractions for PH, HQ and MA changed continuously with increasing air concentrations, suggesting that competing CYP-mediated metabolic pathways favored MA and HQ below 20 ppm and favored PH above 20 ppm. Mean trends of dose-specific levels (µM/ppm benzene) of MA, PH, HQ, and CA all decreased with increasing benzene exposure, with an overall 9-fold reduction of total metabolites. Surprisingly, about 90% of the reductions in dose-specific levels occurred below about 3 ppm for each major metabolite. Using general linear models (GLM) with NS-smoothing functions we detected significant effects upon metabolite levels of gender, age and smoking status. Metabolite levels were about 20% higher in females and decreased between one and two percent per year of life. Also, levels of HQ and CA were greater in smoking subjects. The researchers then extended the GLM+NS models to consider 9 single nucleotide polymorphisms (SNPs) of metabolizing genes, thought to be responsible for production of benzene metabolites [cytochrome P450 2E1 (CYP2E1), NAD(P)H: quinone oxidoreductase (NQO1), microsomal epoxide hydrolase (EPHX1), glutathione-S-transferases (GSTT1, GSTM1 and GSTP1) and myeloperoxidase (MPO)]. After adjusting for benzene exposure, gender, age and smoking status, NQO1*2 affected all five metabolites, CYP2E1 affected all metabolites except CA, EPHX1 affected CA and SPMA, and GSTT1 and GSTM1 affected SPMA. Significant interactions were also detected between benzene
exposure and all four genes and between smoking status and NQO1*2 and EPHX. No significant effects were detected for GSTP1 or MPO. Results generally support prior associations between benzene hematotoxicity and specific gene mutations, confirm earlier evidence that GSTT1 affects production of SPMA, and provide additional evidence that SNPs in NQO1*2, CYP2E1, and EPHX1 affect metabolism of benzene in the human liver. Although all of these effects of metabolism genes and gene-environment interactions were significant, the magnitudes of the effects were small, generally less than two-fold.

This study represents the most comprehensive analysis ever reported of the effects of benzene exposure, metabolism genes, and gene-environment interactions on the levels of benzene metabolites. Overall, the results indicate that benzene metabolism is highly nonlinear with increasing benzene exposure above 0.03 ppm and that current human toxicokinetic models do not accurately predict benzene metabolism below 3 ppm. Since the dose-specific levels of benzene metabolites are much higher at low levels of exposure, current estimates of human risks of leukemia, which were derived from studies of humans and animals exposed to high levels of benzene, may be substantially underestimated at low levels of exposure. The results also indicate that the effects of genes and gene-environment interactions upon benzene metabolite levels are rather small and are dwarfed by the large effects of benzene exposure per se. Finally, the results suggest that GLM+NS models are ideal for evaluating nonlinear relationships between environmental exposures and levels of human biomarkers that have plagued prior studies. These findings are published in Cancer Epidemiology, Biomarkers and Prevention, and in Pharmacogenetics and Genomics.

**Paraoxonases: Biomarkers of susceptibility to environmentally-induced diseases**

University of Washington SBRP

Project Leader: Lucio Costa

The paraoxonases, PON1, PON2, and PON3, play important roles in gene/environment interactions, drug metabolism and susceptibility to vascular and infectious disease. PON2 is expressed ubiquitously and has antioxidant properties. PON1 and PON3 are expressed in the liver and are secreted into the serum, where they are associated with high density lipoprotein particles. PON1 hydrolyzes several organophosphorus (OP) insecticides, oxidized lipids, and other substrates. Many polymorphisms have been identified in PON1, including one that causes an amino acid substitution at Q192R, altering its catalytic efficiency. PON1 levels are highly variable among adults and very low in infants. We coined the term “PON1 Status” to describe a method for simultaneous determination of both PON1 levels and the amino acid present at position 192.

Dr. Costa’s group started a series of investigations aimed at determining the role of PON1 in modulating the toxicity of mixtures of OP compounds. For this purpose, they selected chlorpyrifos oxon (CPO), the active metabolite of chlorpyrifos, and malaoxon (MO), the active metabolite of malathion. Both chlorpyrifos and malathion are widely used insecticides. CPO is metabolized by PON1 in vivo, particularly by the R192 allozyme; MO is not a substrate of PON1, but is metabolized by carboxylesterase (CarE). They found that CPO is a potent inhibitor of CarE, both in vitro and in vivo. When wild-type mice
are exposed to CPO (0.75 mg/kg) and then, after 4 hours, to different doses of MO (25-100 mg/kg), the toxicity of the latter (assessed by measuring inhibition of brain acetylcholinesterase activity) is increased, compared to mice that receive only MO. Such potentiation is due to inhibition of CarE by CPO. A much greater potentiation is observed in PON1 knockout mice, which are unable to detoxify CPO. Indeed, in these animals, CarE inhibition by CPO is greater than that seen in wild-type mice. When comparing hPON1Q192 and hPON1R192 transgenic mice in the same experimental paradigm, the researchers found that the potentiation of MO toxicity was more pronounced in hPON1Q192 animals, because of their lower ability to detoxify CPO. These results indicate that PON1 status can greatly influence the outcome of the interaction between CPO and MO. Further studies in the next year will examine the interaction with MO of diazoxon and paraoxon.

Dr. Costa is also investigating the potential role of PON1 and PON2 in Parkinson's disease (PD). With regard to PON1, they previously conducted a study that did not reveal an association between different PON1 polymorphisms and PD (Kellada et al. 2003). Because of the limitations of genotyping studies, the group is carrying out a study to determine PON1 status in two populations of PD patients and controls. The total number of cases so far is 498, and the controls are 406. A serum sample from each individual was analyzed for PON1 status utilizing a high-throughput two substrate activity/analysis method developed in Dr. Costa's laboratory (Richter et al. 2004), which plots rates of diazoxon hydrolysis against rates of paraoxon hydrolysis. Additionally, arylesterase activity was determined using phenylacetate as a substrate, allowing direct comparison of PON1 levels across all individuals. A preliminary analysis of the data has been carried out. It was confirmed that gene frequencies for PON1 192 did not differ between cases and controls. However, cases had higher arylesterase activity, similar diazoxonase activity and lower paraoxonase activity. Inspection of the arylesterase and paraoxonase data indicated two different slopes for cases and controls. A possible interpretation is that the two substrate sites are subtly different, and rates of hydrolysis of phenylacetate and paraoxon are differentially affected by a different HDL environment. Before any conclusion can be drawn, however, the researchers will analyze additional samples from both populations (one by Dr. Checkoway, the other by Dr. Zabatian), to increase the number of cases and controls toward the original target.

Another objective of Dr. Costa's work is to investigate the potential role of PON2, an enzyme with antioxidant properties that has been reported to be expressed in brain tissue, in modulating the toxicity of the dopaminergic neurotoxicant MPTP. For this purpose his group is in the process of receiving PON2 knockout mice from UCLA. From these, they will initiate their own colony of PON2 knockout mice at the University of Washington. PON2 knockout mice will be initially used to prepare primary cultures of cerebellar granule cells, as described by Giordano et al. (2006), to investigate MPTP toxicity and the role of oxidative stress.
Research Translation

Research Translation activities at the University of Kentucky SBRP
University of Kentucky SBRP
Project Leader: Lindell Ormsbee

While furthering the University of Kentucky Superfund Basic Research Program’s efforts in the disparate areas of technology transfer, broad audiences communication, and government agency partnerships, the Research Translation Core (RTC) has assisted communities exposed to toxins, has influenced local and state decision makers, and has strengthened interactions among stakeholders.

A recent example of the RTC’s positive impact occurred on November 13, 2006, when the UK-SBRP Research Translation Core and the Commonwealth of Kentucky’s Environmental Quality Commission jointly sponsored a public forum on health and the environment. The diverse audience included state policy makers and members of the general public. Regional EPA and ATSDR personnel also were invited to attend. The UK-SBRP presentations included:

- The University of Kentucky Superfund Basic Research Program: Overview and Examples of Research Projects
  - Dr. Bernhard Hennig, Professor of Nutrition and Toxicology, University of Kentucky
- Superfund Community Action through Nutrition - Dr. Lisa Gaetke Associate Professor in Nutrition and Food Science, University of Kentucky

The forum opened lines of communication among public officials, stakeholders, and concerned citizens of Kentucky regarding the Commonwealth’s health and environmental issues. Speakers communicated the availability of resources, as well as the wealth of accessible knowledge and research, for addressing Kentucky’s environmental health challenges. An audience survey was disseminated to attendees. This survey provided valuable feedback to the Research Translation Core regarding attendees’ knowledge of Superfund issues and the perceived efficacy of the meeting itself – both in terms of content and structure. Results of this survey will inform the planning and implementation of future Research Translation events, as well as additional UK-SBRP communication activities.

Another Research Translation Core activity focused on building a partnership with the University of Kentucky’s Clinical and Translational Science Center (CTSC). This relationship will facilitate future connections with citizens of affected communities and will provide an easily accessible network for communication with Area-wide Health Education Centers and rural clinical professional health networks. The CTSC also provides continuing education for rural doctors via web conferencing, which will provide a valuable vehicle for disseminating SBRP research from bench to bedside.

In addition to these efforts, the Research Translation Core has developed a tracking mechanism that evaluates the impacts of the projects and cores through a database. This format enables the RTC to track and evaluate the project- or core-specific translation mechanisms and their impacts. The RTC solicits feedback from a variety of translational mechanisms and generates reports that demonstrate strengths and weaknesses of specific approaches. The reports provided documentation of individual translation activities, as well as the overall success of cumulative translation efforts.

The Research Translation Core, in partnership with the Training Core, has developed a translational workshop for graduate, doctoral, and post doctoral students who work with the SBRP Program. The workshop equips students with the tools to translate research findings in accessible, jargon-free language that can be easily understood by the educated public.
students begin by translating basic biochemistry lessons to audiences with and without scientific backgrounds. The students also gain experience in public speaking and in translating research findings to a broad audiences.

Finally, the RTC is developing a tailored Superfund education website geared toward a variety of audiences, from affected populations to government officials to members of the research community. Due to the nature of UK-SBRP research, the website will focus on the environmental health impacts of chlorinated organics, with a specific focus on PCBs and TCE. With each web page, visitors will be asked a series of questions that will help identify their specific interest and previous knowledge of the topics. These answers will lead visitors to specific pages, which will make additional inquiries that will guide the reader to more defined pages, thus creating a highly customized informational experience for each visitor. An optional participant survey at the end of each visit will provide RTC personnel vital information for improving the site’s efficacy. The model for this website was presented in poster form at the 2006 EPA Science Forum, and the site itself is scheduled for completion in the spring of 2007.

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**Vapor Intrusion**  
Brown University SBRP  
Project Leaders:

The migration of soil vapors into above ground structures (i.e. vapor intrusion) has been the focus of research, debate and public policy for decades. Until the early 1990s, most of the discussion was focused on vapor intrusion of radon, a naturally occurring chemical. More recently, the discussion has shifted to vapor migration of volatile organic compounds (VOCs) into homes and buildings located near hazardous waste sites. This shift has energized a sometimes litigious debate among regulators, members of industry, health experts, and homeowners.

US EPA is responding to the vapor intrusion issue by expending considerable effort developing guidance documents, encouraging a large vapor intrusion database, extensively characterizing sites such as the Raymark Superfund site in Stratford, CT, and actively participating in discussions and conferences focusing on vapor intrusion. State agencies such as NYSDEC and NYSDOH have also made considerable contributions to better understanding of vapor intrusion—using sites such as IBM Endicott, NY as an opportunity for gaining knowledge. Aside from these efforts, research has been also conducted by private industry and academic institutions. Despite these laudable efforts, considerable uncertainty remains with regards to vapor intrusion as a pathway for human health risks. This uncertainty greatly contributes to the ongoing policy debate taking place at national and state levels.

Professor Eric Suuberg (Research Translation Core Director) and Dr. Kelly Pennell (State Agencies Liaison) are involved in regulatory and political discussions about vapor intrusion with our State Agency Partners at the Rhode Island Department of Environmental Management (RIDEM) and the Rhode Island Department of Health (RIDOH). Additional discussions with the Northeast Waste Management Officials Association (NEWMOA) have taken place with regard to upcoming training events for federal and state regulators that will address current vapor intrusion issues. Beyond governmental agencies, the RTC has also engaged with various members of industry about the issue of vapor intrusion at contaminated “legacy” sites.

Through relationships Brown's RTC has built with various agencies and organizations, several vapor intrusion research needs have been identified. One such research area is the effect of geologic heterogeneities on vapor intrusion potentials. Prof. Suuberg and Dr. Pennell recently began investigating vapor phase transport in porous media via a supplemental research
project funded by NIEHS (SBRP). The supplemental NIEHS grant is being used to support a graduate student within the Division of Engineering for the preliminary development of a 3-D vapor intrusion model. The model will be exercised to determine the effect of geologic heterogeneities on pressure-driven transport, and the effect of vapor phase contaminant partitioning on vapor concentrations. These effects, along with the impact of biodegradation are being simulated for a range of scenarios to better understand the impact of vapor intrusion on indoor air environments.

In addition to the activities discussed above, two student projects within the Division of Engineering (one being developed by a group of undergraduates and one being developed by a masters student) are focusing on topics relevant to vapor intrusion. Both of these projects are focused on entrepreneurial approaches to the vapor intrusion problem.