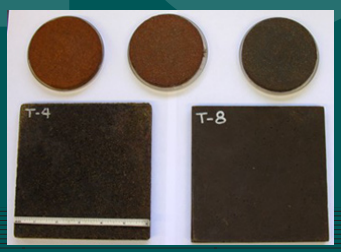
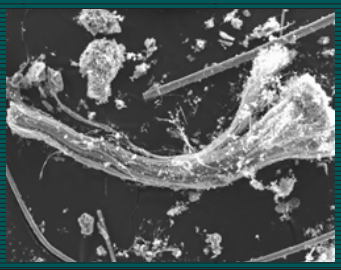
Superfund Basic Research Program

Research Briefs 2007





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SBRP Research Briefs

Since 1997, the Superfund Basic Research Program has reached out to the stakeholder community on a monthly basis, electronically distributing summaries of major ongoing research projects. As of December, 2007, 156 Research Briefs have been produced and distributed.

The Research Briefs are sent to over 4.600 individuals in 64 countries. Over half of the subscribers are USEPA employees – many of whom forward the documents to their co-workers. Other subscribers include:

- Government employees in 31 federal agencies and 42 state agencies
- Staff members of the National Academies of Science, Engineering, and Medicine
- Researchers and students at 145 universities
- Environmental professionals at over 230 remediation/engineering firms
- Members of 48 not-for-profit organizations

As illustrated by the following excerpts from readers' comments, the Research Briefs are well received by the diverse audience:

- "We find the *Research Brief* series so useful that I save and catalog each one as part of the monthly new additions to the California Water Boards' Joint Intranet Technical Library."
  ***Ed Wosika**, Assoc. Eng. Geologist; Land Disposal Unit; California State Water Resource Control Board*

- "I just read your research brief and wanted to commend you for such excellent work!…I think most project managers at USEPA get the *Research Briefs*, but I typically forward the research briefs to friends and other colleagues in and outside USEPA, too. You'd be surprised how many folks want to learn the latest information and take whatever measures they deem appropriate. Please keep up the good work and know that your findings are spread far and wide!"
  ***Shelley Brodie**, USEPA; Superfund Remedial Project Manager*

- "We found your documents on the web and would like to be added to your e-mail list to learn more about environmental issues. We are a small group (Brooklyn NY) that formed to get better facilities at the neighborhood park and has evolved into an environmental group. We are hoping to pass this acquired knowledge on to other park users."
  ***Laura and Mike Hofmann**, Barge Park Pals*

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Research Brief 145: Arsenic Affects All Five Steroid Receptors

Release Date: 01/03/2007

Background: Epidemiology studies of populations exposed to low doses of arsenic have documented increased risks of numerous and diverse human health effects including several different cancers, type 2 diabetes, vascular disease, cardiovascular disease, and reproductive and developmental problems. The mechanism(s) underlying these various health effects is unknown. Knowledge of how long-term exposure to low, environmentally relevant doses of arsenic initiates processes that lead to adverse health effects is critical to evaluating overall disease risk of people who are exposed to arsenic in drinking water.

Advances: Dr. Joshua Hamilton's group at the Dartmouth College SBRP first reported that arsenic is a potent endocrine disrupter, blocking the ability of the glucocorticoid hormone to signal through its hormone receptor, the glucocorticoid receptor (GR). Dr. Hamilton, in collaboration with Dr. Jack Bodwell of the Dartmouth Medical School, conducted a follow-up study investigating arsenic's impacts on other hormone receptors. They focused on three other closely related members of the steroid receptor family - the progesterone receptor (PR), androgen receptor (AR) and mineralocorticoid receptor (MR). The hormones they interact with to elicit gene expression (i.e., progesterone, testosterone and the mineralocorticoid hormones, respectively) that regulates a wide range of biological processes and Dr. Hamilton believes that disruption of their normal function could explain, in large part, how arsenic can influence so many disease processes in so many diverse tissues.

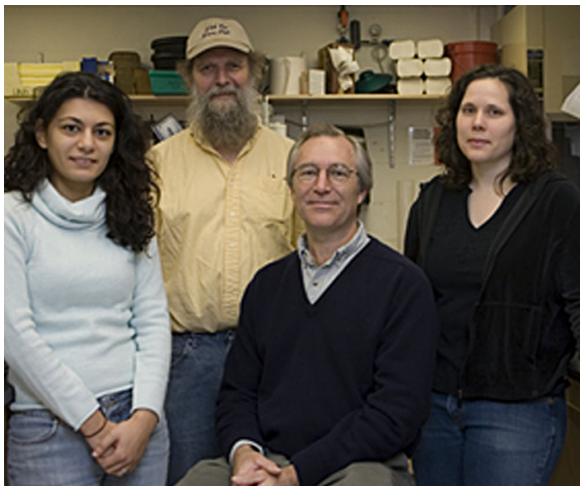
Dr. Hamilton's group used a cell culture model to evaluate steroid receptor responses to arsenic exposure, working with EDR3 rat hepatoma cells that lack endogenous expression of these receptors but which exhibit normal hormone-inducible gene expression when each of these receptors is individually re-expressed in the cells by transfection of the appropriate genetic construct. The researchers exposed these engineered cells to low levels of arsenic and then assessed its effects on levels of receptor-induced gene expression. They found that like the GR, the other steroid receptors tested all showed a biphasic response: low arsenic levels (0.1-0.7 μM, 7.5-50 ppb) stimulated hormone-induced transcription, while slightly higher but still non-toxic levels (1-3 μM, 75-225 ppb) were inhibitory. In a separate study (manuscript in process), the researchers found similarly that the estrogen receptor (ER) displays the same basic pattern of response. Thus, all five of the steroid receptors responded to arsenic disruption the same way. This unusual dose-response suggests that arsenic might have very different biological effects at the lower and higher doses.

The Dartmouth finding that all the receptors in the steroid receptor family respond similarly to arsenic exposures suggests that arsenic alters some common target or process in the steroid receptor mechanism of action. Using the GR as a model for other steroid receptors, Dr. Hamilton's group evaluated a number of possible mechanisms:

- Arsenic could modify cellular levels of hormones.
- Arsenic could alter cellular levels of receptors.
- Arsenic could activate the receptors by mimicking the natural hormone.
- Arsenic could impact the steps immediately downstream of nuclear translocation and receptor activation, i.e., dimerization and DNA binding.
- Arsenic could alter the ability of hormone-activated steroid receptors to bind to DNA.

Dr. Hamilton's current data, in conjunction with previous studies by this group, clearly demonstrate that none of these mechanisms is responsible for steroid receptor-mediated response to low-dose arsenic. It is likely, based on these results to date, that the mechanism for arsenic's effects involves a shared pathway or regulatory machinery that directly affects hormone-receptor mediated gene transcription.

Significance: These results suggest that many other nuclear hormone receptors may be affected similarly by arsenic. The researchers are currently investigating whether arsenic also affects other members of the larger nuclear hormone receptor super-family, such as the receptors for thyroid hormone or retinoic acid, which are more distally related to the steroid receptors but share some common regulatory machinery. Given the myriad of functions that these receptors and their hormones modulate in normal human biology, as well as the many diseases in which they have been shown to play a fundamental role, Dr. Hamilton hypothesizes that this is one of the major pathways by which arsenic increases the risk of the growing list of diseases associated with arsenic exposure.



Left to right: Dartmouth researchers Athena Nomikos, Jack Bodwell, Josh Hamilton, Julie Gosse

These results also suggest that there may be human health effects at drinking water levels of arsenic at or below the current U.S. drinking water standard of 10 ppb since these effects were observed at levels far below 10 ppb in cell culture and at or below this level in several *in vivo* models.

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To learn more about this research, please refer to the following sources:

Bodwell, Jack, Julie A. Gosse, Athena P. Nomikos, and Joshua W. Hamilton. 2006. Arsenic disruption of steroid receptor gene activation: complex dose-response effects are shared by several steroid receptors. Chemical Research in Toxicology (http://pubs.acs.org/journals/crtoec/index.html). 19(12):1619-1629.

Research Brief 146: Groundwater Contamination by Perchlorate from Brines

Release Date: 02/07/2007

Background: In the late 1990's, Region IX of the US EPA approached Dr. Martyn Smith, Program Director of the University of California at Berkeley Superfund Basic Research Program, concerning the recently discovered perchlorate contamination of groundwater throughout the western region. Ammonium perchlorate, the major component of solid rocket fuel, was used extensively in the aerospace industries. Because perchlorate was not a regulated contaminant, wastewaters were frequently released to the environment.

The situation encountered with perchlorate is not unique. There are numerous other instances of subsurface contamination caused by the release of concentrated brines containing contaminants at levels that far exceed water quality guidelines. Acid mine drainage, landfill leachates and nuclear fuel reprocessing solutions are some of the industrial brines that are known to impact groundwater resources.

Dr. James Hunt at U.C. Berkeley initiated research to understand the processes that control the movement of brines, such as perchlorate, in the subsurface. He demonstrated that the density contrast with ambient groundwater will cause concentrated perchlorate solutions to sink through an aquifer until pooling on a lower-permeability confining layer. Density differences will then cause the brine to migrate and diffuse into the lower-permeability confining layer. Perchlorate then slowly diffuses into the flowing groundwater leading to the creation of a long-term source zone. This scenario mirrors the formation of concentrated source zones that are common in aquifers contaminated with dense nonaqueous phase liquids (DNAPLs) and both situations represent difficult remediation challenges.

Advances: A fundamental understanding of brine transport and recovery is needed in the design of optimal remediation systems using technologies that involve the injection of dense water-miscible fluids into the subsurface to accomplish contaminant destruction, isolation, or removal. Brines are miscible with water - but are more dense and more viscous than ambient groundwater. These property differences can dominate (a) transport in the subsurface, (b) release of contaminants associated with brines into groundwater, or (c) delivery and recovery of remedial fluids. While the freshwater-seawater interface in groundwater systems has been extensively studied, the brines identified in this work have much greater contrasts in density and viscosity than encountered at freshwater-seawater interfaces.

Dr. Hunt's group compiled previously published data on vertical transport of brines in porous media and compared these with a generalization of an existing stability analysis. The available data were not sufficient to test the stability analysis and quantify brine dispersion during one-dimensional vertical displacements. The researchers conducted additional one-dimensional laboratory-scale experiments to examine the downward displacement of water by brine and brine displacement by water. Under downward flow conditions, gravitational and viscous forces work against each other and these tests were specifically designed to examine the mixing processes. The researchers conducted nearly 400 tests, using calcium chloride and potassium chloride as experimental brines. The resulting data set and analysis demonstrate the applicability of the stability analysis to predict conditions under which brine dispersion will be enhanced by unstable displacements and suppressed by stable configurations.

Significance: Brines have not been fully appreciated as source terms for groundwater contamination or for constraining the delivery and recovery of remediation solutions. Because brines are completely miscible with water, the initial expectation is that brines could be displaced by groundwater flow. On the other hand, brine density and viscosity dominate the transport of the brines and associated contaminants within aquifers and into less accessible subsurface environments. Brine dispersion as quantified during vertical transport in this research can be used to approximate brine emplacement in aquifers. Dr. Hunt provided the first synthesis of existing data for one-dimensional vertical displacements, either upwards or downwards. Quantification of these transport processes is essential in understanding the long term source of contaminants that will be present in groundwaters requiring treatment and in the design of delivery and recovery systems for in situ remediation fluids.

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To learn more about this research, please refer to the following sources:

Flowers, Tracey C. and James R. Hunt. 2007. Viscous and gravitational contributions to mixing during vertical brine transport in water-saturated porous media. Water Resources Research (http://www.agu.org/wrr/). 43(1):Art.No.W01407.

Flowers, Tracey C. and James R. Hunt. 2000. Long-term release of perchlorate as a potential source of groundwater contamination. Chapter 14 in: Perchlorate in the Environment. Kluwer/Plenum, NY.

Research Brief 147: Microbial and Photolytic Transformations of Environmental Contaminants

Release Date: 03/07/2007

Background: Researchers at the Duke University SBRP are studying the effects of microbial and photolytic processes on transformations of polycyclic aromatic hydrocarbons (PAHs) and organophosphate pesticides (OPs). Their goals are to better understand natural transformation processes occurring at Superfund sites and to develop systems to accelerate the destruction of chemical contaminants.

Much of this research focuses on intermediate products of the degradation processes. These products are of particular interest for several reasons:

- Intermediate products of microbial and photolytic processes can be more bioavailable and/or more toxic than the parent compounds. It is important to acknowledge that removal of a parent compound alone may not be the best measure of site cleanup.
- Intermediate products may affect other microorganisms involved in bioremediation of the parent compound or other contaminants.
- Combined microbial and photolytic processes may be synergistic. The Duke researchers are working to assess biodegradability of photolytic end products and photodegradability of biodegradation end products. For example, high molecular weight PAHs tend to be recalcitrant to microbial degradation but are susceptible to photolytic degradation. Thus, combined photochemical and microbial treatment could be used to transform high molecular weight PAHs to more biodegradable forms.

Advances: Drs. Andrew Schuler and Karl Linden are studying 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 focusing on degradation of the sulfur-containing PAH dibenzothiophene (DBT) by a mixed bacterial culture. They monitor: a) DBT degradation rates; b) intermediate product formation; c) microbial population dynamics of 10 taxonomic groups using quantitative PCR (qPCR) assays; and d) changes in toxicity via 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, degradation proceeded steadily and over 90% DBT reduction was achieved. The Schuler/Linden research team determined that the formation of toxic intermediate products is an important concern, as toxicity initially doubled and stayed at relatively high levels even after the DBT was degraded. 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 may be of particular interest in the degradation of DBT.

The researchers also investigated photolytic process. They constructed a simulated natural sunlight system to study natural photolysis of the PAHs fluorene (FLU), dibenzofuran (DBF) and DBT alone and in mixtures. They observed no direct photolysis during 8 hours of exposure to natural sunlight, but did find that there was decay in the presence of hydrogen peroxide, suggesting that oxidative degradation is a significant environmental pathway. The researchers studied UV-based oxidative processes (UV photolysis and hydrogen peroxide assisted photodegradation) of the same group of PAHs and found a synergistic effect during photolysis with higher degradation rates within the mixture as compared to photodegradation as single components in an aqueous solution.

The researchers have also determined the kinetics and mechanisms of the photodegradation of the organophosphate pesticides chlorpyrifos, parathion, and diazinon under engineered and natural UV decay conditions. They have developed fundamental parameters of UV-based decay 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.

Significance: 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.

This work is yielding important information regarding the fundamental processes affecting toxic exposures in natural systems. Very little work has been done on the interaction between microbiological and photodegradation processes, yet this combination may be particularly important in estuary systems, where chemicals suspended in the water column are exposed to microbes and to sunlight, and it may hold promise for the development of new engineered systems for remediation.

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To learn more about this research, please refer to the following sources:

Shemer, Hilla and Karl G. Linden. 2007. Aqueous photodegradation and toxicity of the polycyclic aromatic hydrocarbons fluorene, dibenzofuran and dibenzothiophene. Water Research (http://www.elsevier.com/inca/publications/store/3/ 0/9/index.htt). 41(4):853-61.

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Research Brief 148: Dual Role for Vitamin C in Cr(VI) Toxicity

Release Date: 04/04/2007

Background: Hexavalent chromium [Cr(VI)] is listed by the EPA as a known human carcinogen, yet under normal physiological conditions it is unreactive toward DNA and other important biological macromolecules. Cr(VI) must be reduced to trivalent chromium [Cr(III)] to induce biological damage. The molecular mechanisms underlying the cytotoxic, mutagenic and carcinogenic effects of Cr(VI) involve complicated intracellular chemistry, multiple intracellular targets and multiple pathways.

Reduction of Cr(VI) to Cr(III) can occur via non-enzymatic reactions with cysteine and glutathione, but in the target tissues of chromate toxicity, ascorbate (reduced vitamin C; Asc), a two-electron donor, is the primary reducer of Cr(VI). Interestingly, while human lung tissue has ~1.3 mM Asc, human cells in culture contain no or barely detectable amounts Asc - simply because the majority of synthetic media formulations do not include vitamin C. As a result, mechanisms of Cr(VI) genotoxicity in cultured cells have not been studied under physiologically relevant conditions.

Advances: Dr. Anatoly Zhitkovich of the Brown University SBRP is conducting basic research to identify biomarkers of genetic damage following exposure to Cr(VI). As part of this work, his team developed a method to load human and other cultured cells with Asc. They pre-treat cells with dehydroascorbic acid, the oxidized form of vitamin C, which readily enters cells and is then reduced to Asc, rapidly restoring physiological concentrations of Asc.

Dr. Zhitkovich's research group used this test system to examine intracellular responses of human lung cells following exposure to Cr(VI). They found that restoration of physiological Asc levels in these cells strongly increased chromosomal damage following even low-dose Cr(VI) exposure in an Asc-concentration-dependent manner.

They investigated the effect of cellular Asc on the genotoxic potential of Cr(VI) by comparing the numbers of biomarkers of DNA double strand breaks (DSB) in cells preloaded with a range of Asc concentrations. They found that preloading of primary human bronchial epithelial cells with Asc greatly enhanced the ability of low doses of Cr(VI) to cause DSB, increasing the yield of biomarker-containing cells by 8-20-fold. Assessment of the impact of cellular Asc on repair of chromosome breaks revealed a more than 10-fold increase in the levels of unrepaired DSBs in Asc loaded cultures.

The researchers conducted studies to see if increased genetic damage due to cellular Asc results from the changes in the types of DNA lesions and/or altered cellular responses to the same Cr-DNA damage. They added Asc to Cr-exposed test cultures after sufficient time for the completion of Cr(VI) reduction in Asc-free cells. No effect on the formation of DSB or the amount of unrepaired chromosomal damage was detected when Asc was introduced following exposure and reduction of Cr(VI). This indicates that Asc must be present in cells during Cr(VI) reduction - Asc does not change biological responses to Cr-DNA damage that is already formed. Therefore, the targets of Asc are Cr(VI) metabolism and resulting DNA damage rather than cellular damage response mechanisms.

Dr. Zhitkovich's group also investigated whether the increase in chromosome damage by Asc was a direct result of the formation of highly toxic DNA lesions or if it was caused by cellular responses to Asc-mediated Cr-DNA damage. They focused on mismatch repair (MMR) proteins, which ensure genomic stability by correcting mutagenic mismatches arising during DNA replication. The researchers determined that suppression of MMR essentially eliminated the formation of DSB. Thus, unrepaired chromosomal breaks are apparently caused by MMR proteins mis-processing Asc promoted Cr-DNA damage.

They also examined apoptotic responses following Cr exposure using three biochemical markers of apoptosis. They found that the presence of Asc during the reduction of Cr(VI) enhances Cr-induced apoptosis. Induction of apoptotic responses to many types of DNA damage is usually mediated by increased expression of p53 dependent genes. Dr. Zhitkovich's group examined the role of p53 in Cr(VI)-associated cell death and found that depletion of p53 had no significant effect on the levels of Cr(VI)-induced cytotoxicity, both in the presence and absence of intracellular Asc. These findings indicate that cellular vitamin C should enhance the ability of Cr(VI) to select for mismatch repair-deficient cells in a p53- independent manner and are in agreement with a low incidence of p53 mutations in chromate-associated human lung cancers. The majority of Cr-induced human lung carcinomas have inactive mismatch repair, which was proposed by Dr. Zhitkovich to result from selective outgrowth of MMR-deficient cells due to their resistance to killing by Cr(VI).

Significance: Vitamin C has been used as an antidote in industrial accidents and other instances when large amounts of chromium are ingested. It works by rapidly reducing Cr(VI) to Cr(III), which enters cells much slower than Cr(VI), thus reducing chromium's intracellular toxic effects. In contrast, Dr. Zhitkovich's research demonstrates that cellular Asc acts as a potent amplifier of Cr(VI)'s genotoxic activities. Thus, Asc plays a dual role in Cr(VI) toxicity: protective outside the cell and potentiating inside the cell.

This work is important as it identifies the mechanism of Asc-induced increases in DNA breakage, but also because it discovered that increasing Asc concentrations generates progressively more mutations and DSB - revealing the genotoxic potential of otherwise nontoxic doses of Cr(VI). When combined with Asc, Cr(VI) caused genetic damage in cells at doses four times lower than current federal standards and these findings might have potential policy implications.

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To learn more about this research, please refer to the following sources:

Reynolds, Mindy and Anatoly Zhitkovich. 2007. Cellular vitamin C increases chromate toxicity via a death program requiring mismatch repair but not p53. Carcinogenesis (http://carcin.oupjournals.org/). 28(7):1613-1620.

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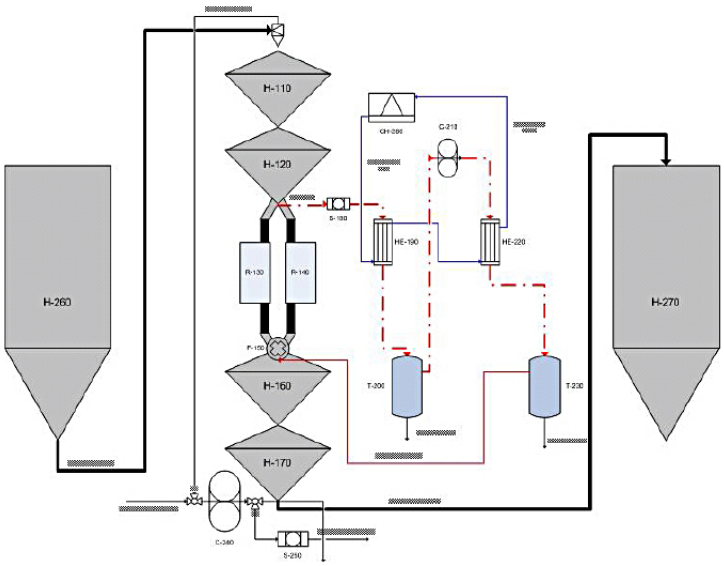
Release Date: 05/02/2007

Background: Many technologies are applied for hazardous waste site remediation including soil vapor extraction, catalytic oxidation and other thermal methods. These methods are costly, time-consuming and can generate concerns within local communities. Carbon adsorbents are prevalent in gas and liquid cleanup operations at Superfund and other hazardous waste sites, particularly for dilute waste streams. Granular activated carbon (GAC) readily adsorbs volatile organic compounds (VOCs), nitrogen oxides (NOx), and sulfur oxides (SOx) from the gas phase. As adsorption progresses, the available adsorption sites diminish and the efficiency of the GAC decreases. To maintain high adsorption efficiencies, the saturated carbon adsorbents are generally removed and transported to landfills for disposal. Fresh carbon adsorbents must be purchased periodically to replace the spent adsorbents. The cost of this GAC changeout is generally prohibitive unless the waste stream is extremely dilute. Alternatively, the used GAC may be reactivated by liberating the adsorbed chemicals, restoring the available adsorption sites on the carbon, thereby concentrating the adsorbed chemicals into a waste stream that is more easily disposed. In some cases, the previously "wasted" chemicals can even be recycled.

Dr. Chang Yul Cha, of the CHA Corporation, discovered that microwaves provide an effective means of reactivating carbon adsorbents, and the process can be implemented on site. The CHA microwave-based GAC regeneration process is designed to desorb and capture a wide variety of both chlorinated and non-chlorinated VOCs. As a result, the GAC can be reused and potentially valuable chemicals can be recycled. Even if the recovered chemicals have little value, the volume of waste is significantly reduced, simplifying disposal. Because the cost of carbon change out can be reduced or eliminated, it is economically feasible to use GAC as an alternative to catalytic oxidation for concentrated waste streams.



Process Flow Diagram for 50-kg/hr Mobile Microwave Unit

Advances: With Phase I and Phase II Small Business Innovation Research (SBIR) funding from the SBRP, Dr. Cha conducted extensive design, laboratory and scale up experiments plus field tests at McClellan Air Force Base. Most recently, the CHA Corporation designed, constructed and tested a field-ready mobile microwave carbon reactivator. The mobile microwave reactivating unit constructed by the CHA Corporation utilizes two 25-kg/hr microwave generators to regenerate carbon at an overall rate of 50-kg/hr. Carbon hoppers are arranged in an airlock configuration, to allow for continuous processing of large amounts of activated carbon. The system is compact enough to mount on a trailer, making it simple to regenerate carbon at any site.

In May 2006, Dr. Cha transported the 50-kg/hr mobile unit to McClellan Air Force Base for a 2-week field demonstration. The Air Force is currently evaluating the commercial viability of the CHA microwave technology in conjunction with carbon adsorption as an alternative to the existing catalytic oxidation systems used at soil vapor extraction (SVE) sites containing chlorinated solvents. Field data were confirmed with laboratory testing of GAC saturated with toluene. Results demonstrated removal efficiencies of 96 - 99.5%, depending on the amount of microwave power applied.

In addition, Dr. Cha is investigating the applicability of the CHA microwave system to regenerate GAC saturated with tetrachloroethylene (PERC). Dr. Cha placed a small adsorber on site at a dry cleaning facility to collect PERC from the exhaust air stream. The stream had a very low PERC concentration (less than 5 parts per million by volume). The test began with 37.2 kg of fresh GAC adsorbent. After saturation with PERC, the weight was 49.5 kg. The carbon was then regenerated with the CHA microwave system, yielding 38.4 kg of regenerated GAC (about 95% efficiency). A total of 6 liters of solvent was recovered during regeneration of the GAC and returned to the dry cleaner. Preliminary characterization of the solvent recovered from the dry cleaner indicated a specific gravity of 1.57, very close to the specific gravity of 1.6 given on the Material Safety Data Sheet (MSDS). The solvent was added back into the solvent tank of a dry cleaning unit and used in the dry cleaning process. The recovered solvent performed qualitatively the same as the fresh solvent.

Mobile Unit at McClellan Site OUD

Significance: Dr. Cha estimates the total capital cost of an installed 50-kg/hr microwave onsite reactivation system to be $291,000. This cost is comparable to a catalytic oxidation system capable of treating 850-m3/hr contaminated air from soil vapor extraction.

Total carbon reactivation cost is $0.60 per kg for the CHA Corporation's integrated 50 kg/hr system. The conventional GAC change-out cost including transportation and offsite disposal is approximately $4.73/kg.
This difference in the operating costs could result in recovery of capital costs within one year.

There is a high potential for economic and environmental benefits from recycling of PERC used by the dry cleaning industry. Currently, large amounts of PERC are lost in the air exhausted from dry cleaning processes. Most of this PERC could be recovered with the installation of a GAC adsorber and by utilizing the CHA microwave technology to regenerate the GAC and recover the adsorbed PERC.

Overall, the benefits of this technology are: (1) the cost advantage compared to remediation systems which rely on GAC disposal; (2) vastly concentrated (reduced) waste streams, which can be more easily managed to prevent environmental contamination; (3) capture and potential recycling of valuable solvents; and (4) improved community acceptance compared to thermal technologies and others that produce higher volume waste streams and/or a greater potential for environmental contamination.

Dr. Cha is eager to apply the microwave technology at other sites around the country. Please see the CHA Corporation web site for additional information: http://www.chacorporation.com/.

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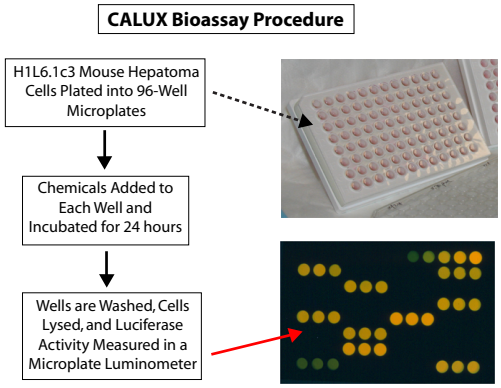
Research Brief 150: A New USEPA SW-846 Method 4435 for HAH Analysis

Release Date: 06/06/2007

Background: Accurate identification and quantitation of dioxin-like halogenated aromatic hydrocarbons (HAHs) in environmental matrices are critical for site assessment, risk assessment, and environmental monitoring. Although sophisticated cleanup and instrumental analytic procedures are available to separate, identify, and quantitate individual HAHs, these procedures require specialized equipment and are both costly and time-consuming. A US Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation (SITE) report (http://www.epa.gov/ord/SITE/reports/540r05001/540r05001.pdf) states that the gas chromatograph/mass spectrometer (GC/MS) analysis of 209 demonstration samples cost approximately $1100 per sample and took approximately 8 months.

An inexpensive and rapid screening assay capable of detecting and estimating the relative biological/toxic potency of complex mixtures of dioxin-like HAHs in numerous matrices would greatly facilitate exposure and epidemiological analysis and screening for HAHs. With support from the Superfund Basic Research Program, Dr. Michael S. Denison at the University of California, Davis and Dr. George C. Clark of Xenobiotic Detection Systems (XDS), have developed a rapid and inexpensive recombinant cell bioassay system for the detection and relative quantitation of dioxin-like chemicals in sample extracts that is amenable for large scale screening analysis.



In May 2007, the EPA's Office of Solid Waste (OSW) Organic Methods Workgroup formally reviewed and approved the draft Method 4435 for inclusion into *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, also known as SW-846. The XDS-Chemically-Activated Luciferase gene expression cell bioassay system (XDS-CALUX®) for dioxin-like chemicals, will soon be published and will be posted under "new methods" on the SW-846 website (http://www.epa.gov/epaoswer/hazwaste/test/sw846.htm).

Advances: Drs. Denison and Clark and their laboratories designed the XDS-CALUX® bioassay on knowledge of the molecular mechanism of toxicity of dioxin-like HAHs. They used recombinant technology to place the firefly luciferase gene under the control of the Ah Receptor (AhR) and inserted it into a mouse cell line. The bioassay uses the cell line to detect the presence and level of dioxin-like HAHs in a sample as these compounds bind to the AhR, which then localizes in the nucleus of the cell and binds to dioxin responsive elements (DRE) inducing expression of firefly luciferase. The enzymatic activity of luciferase leads to the production of light that is easily measured. Dioxin-like HAHs present in extracts of environmental matrices are determined following sample processing methods. The assay responds in a time-, dose-, and AhR-dependent and chemical-specific manner. The XDS-CALUX® bioassay is sensitive to <1 part per trillion for dioxin/furan and polychlorinated biphenyls (PCB) analyses. The bioassay is applicable to: biological matrices including blood (whole serum and extracts), breast milk, and tissues extracts; environmental matrices including soil and sediment, ash, and pulp and paper; and food matrices including animal fats (oil and fats), milk and butter, and animal feeds.

Drs. Denison and Clark have been working to fine-tune the assay for nearly 10 years and have made it available to the research and regulatory communities. Accordingly, the use of the patented technology (US patents 5,854,010 and 6,720,431) has been spread throughout the world:

- **1999** - 500 tons of feed contaminated with approximately 50 kg of PCBs and 1 g of dioxins were distributed to animal farms in Belgium. Shortly afterwards the Belgian government licensed the XDS-CALUX® bioassay for the detection of dioxin-like chemicals in order to protect their residents and food supply.
- **June 2000** - XDS licensed the Hiyoshi Ecological Services Company (Hiyoshi Corporation of Japan) to use their technology. Since then, Hiyoshi has become an international leader in using the XDS-CALUX® bioassay and has contributed to licensing of this valuable technology to other countries in Taiwan, China, Vietnam, and Laos.
- **October 2001** - the Food and Drug Administration (FDA) Office of Regulatory Affairs, Arkansas Regional Laboratory signed a licensing agreement to use the XDS-CALUX® bioassay for investigation as a new technology in the detection of dioxin-like compounds.
- **August 2002** - the Belgian government's accreditation service certified XDS' patented bioassay technology as a valid and accurate method for screening detection of chlorinated dioxins/ dibenzofurans and PCBs. As a result of the certification, the XDS-CALUX® bioassay licensed by the Belgian government is now an accepted method throughout the European Union for the screening detection of dioxins and PCBs in food products, and has been widely used for the analysis of biological and epidemiologic studies.
- **January 2005** - the XDS-CALUX® bioassay was selected by the government of Poland as a screening tool for detection of chemical contamination of their feed and food supply.
- **May 2005** - XDS was invited to participate in the US EPA's SITE Program. The XDS-CALUX® bioassay was evaluated in the program study and was an outstanding performer.
- **September 2005** - the Minister of the Environment, Japan, promulgated a ministerial notification designating the XDS-CALUX® bioassay as a concrete simplified method in accordance with the law concerning special measures against dioxins.
- **May 2007** - the EPA OSW Organic Methods Workgroup officially approved the XDS-CALUX® bioassay for publication in SW-846.

Significance: The XDS-CALUX® bioassay analyses costs are 40%-70% lower than GC/MS analyses. In the EPA SITE study, the XDS-CALUX® analysis took 1.5 months to analyze 209 samples vs. 8 months for the GC/MS analysis. These substantial savings make XDS-CALUX® an excellent screening tool and give researchers, laboratories, and government agencies the option to reduce the use of expensive GC/MS analyses in conjunction with the XDS-CALUX® results to estimate if dioxin levels are above client-established threshold levels.

The EPA OSW Organic Methods Workgroup, by accepting the method for publication in SW-846, approves the bioassay for use in conducting the evaluations and measurements needed to comply with the Resource Conservation and Recovery Act (RCRA). Because other countries look to the US EPA for approval or validation of methods before they are implemented, the inclusion of XDS-CALUX® in SW-846 is a very important step in getting the technology used throughout the US and world.

Development of the XDS-CALUX® technology was funded in part by Superfund Basic Research Program Grant ES04699, and by NIEHS Small Business Innovation Research Grants ES 08372-01 and ES 08372-03.

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To learn more about this research, please refer to the following sources:

Clark, George C., J. Orelien, John D. Gordon, Andrew C. Chu, Michael D. Chu, and Michael S. Denison. 2005. Mathematical model predictions of GC/MS data for dioxin contaminated environmental samples using CALUX® by XDS. Organohalogen Compounds. 67:50-53.

Windal, Isabelle, Michael S. Denison, Linda S. Birnbaum, Nathalie Van Wouwe, Willie Baeyens, and Leo Goeyens. 2005. Chemically activated luciferase gene expression (CALUX) cell bioassay analysis for the estimation of dioxin-like activity: critical parameters of the CALUX procedure that impact assay results. Environmental Science and Technology (http://pubs.acs.org/journals/esthag/). 39(19):7357-7364.

Research Brief 151: The Dartmouth Community Outreach Core Facilitates Change in New Hampshire Lead Laws

Release Date: 07/03/2007

Background: The removal of lead from gasoline in 1973 and the resulting reduction in blood lead levels are generally perceived as public health success stories. However, in many areas of the country, exposure to lead is still a concern due to lead-based paints found in old housing. Dust and soil contamination most frequently occurs when lead-based paints are peeled, worn, or sanded in or around housing. Children are most commonly exposed to lead through chronic ingestion of lead-contaminated dust. Lead poisoning is a serious environmental health issue in communities such as Manchester, New Hampshire, where the housing stock is old. In 2000 a Manchester, NH child died of lead poisoning.

High blood lead levels (>70 µg/dL) can cause serious health effects, including seizures, coma, and death. Blood Lead levels as low as 10 µg/dL have been associated with adverse effects on cognitive development, growth, and behavior among children aged 1-5 years.

For the past five years, Nancy Serrell and her staff at the Dartmouth College SBRP Community Outreach Core have been working with the Manchester Health Department, The Way Home (a housing advocacy group), and the Greater Manchester Partners Against Lead Poisoning to improve measures to prevent lead poisoning in New Hampshire. Ms. Serrell and her team have initiated and supported diverse activities to facilitate awareness and collaboration among community groups, health departments, non-profit organizations, and family medical practices.

Peeling paint

Advances: The work was initially funded through two EPA pilot grants and evolved as partnerships expanded into the Manchester-Dartmouth Partnership for Health (MDPH). With additional funding from SBRP, the MDPH worked with a coalition of stakeholders in a series of activities that led to promulgation of new legislation that promotes primary prevention of childhood lead poisoning:

Collaboration with the Vermont Law School. Ms. Serrell approached the Vermont Law School to find collaborators to help research and produce a report on the essential characteristics of a good lead law. Together, the Vermont Law School and the MDPH produced *State Legislation Addressing Prevention of Childhood Lead Poisoning: A Policy Report for the Greater Manchester (NH) Partners Against Lead Poisoning* (http://www.dartmouth.edu/~toxmetal/Legislative%20Report-GMPALP.pdf) and distributed the report to their partners in New Hampshire as well as in Vermont, where stakeholders were beginning a review of Vermont's lead laws.

Convening of a "Call to Action" community meeting. The MDPH produced a white paper, "A Call to Action: Eliminating Lead Poisoning in Manchester," and sponsored a community breakfast for foundations, the banking community and other stakeholders. The goal of the meeting was to propose several "action steps" and to foster new partnerships. Following this meeting, two New Hampshire foundations agreed to fund a broad-based Lead Poisoning Prevention Collaborative to coordinate statewide efforts to tackle lead poisoning.

Collaboration with not for profit and government organizations. In fall of 2006, the Dartmouth Outreach Core submitted a proposal to make lead poisoning prevention an "Action Step" priority for the New Hampshire Children's Advocacy Network (NH CAN), a coalition of nearly 200 state organizations committed to children's issues. NH CAN members voted to adopt the Action Step as a priority for the 2007 legislative session. As part of this process, Dartmouth's outreach team was trained by the FrameWorks Institute (http://www.frameworksinstitute.org/) in effectively using linguistic framing to influence public discourse. In December 2006, Ms. Serrell was appointed to the Governor's Task Force on Lead Poisoning Prevention, which was asked to review the state's current laws and regulations and to recommend changes.

Development of a Quality Improvement Toolkit for medical professionals. Though the U. S. Centers for Disease Control recommends that medical practices in communities with a predominance of pre-1950s housing screen all one and two-year olds for lead poisoning, screening rates in Manchester fall

short of this guideline. In partnership with five city medical practices, the MDPH is working to address this issue. The project began with an audit of medical charts to identify whether practices were screening children appropriately and lunchtime conversations with medical practitioners to identify barriers to screening. New partners (the NH Childhood Lead Poisoning Prevention Program and the University of New Hampshire) have joined the project, and the collaborators are designing and testing an online Lead Screening and Management Quality Improvement Toolkit that will include a streamlined chart audit tool, Grand Rounds tutorials and patient materials in several languages.

Members of the Manchester-Dartmouth Partnership for Health, from left to right: Bethany Fleishman, Outreach Assistant, Dartmouth Toxic Metals Research Program; Mary Sliney, director of The Way Home; Nancy Serrell, Director for Outreach and Translation, Dartmouth Toxic Metals Research Program; Fred Rusczek, former Director of the Manchester, NH Health Department.

Participation in a Vermont initiative to research funding strategies for lead poisoning prevention. Ms. Serrell and Bethany Fleishman (Outreach and Translation Assistant) were invited by the Vermont Attorney General and Vermont Commissioner of Health to participate in a statewide "Get the Lead Out of Vermont" initiative. They participated in a subcommittee that researched and reported on funding strategies for lead poisoning prevention.

Significance: Inspired at the "Call to Action" community meeting co-sponsored by the Dartmouth Outreach Core and the Manchester Health Department, Senator Betsie DeVries (D) proposed legislation to help protect children from lead exposure. New Hampshire SB 176, which was passed by the NH Senate and House on June 17, 2007, incorporates the key recommendations of the Governor's Task Force on Lead Poisoning Prevention. The new bill:

1. Lowers the blood lead level that determines when a child is lead poisoned from 20 µg/dL to the action level (10 µg/dL) set by the U.S. Centers for Disease Control and Prevention;
2. Authorizes the Commissioner of the NH Department of Health and Human Services to inspect other units of a multi-unit dwelling when a child has been found to be lead poisoned in one of the units;
3. Extends the period of time that interim controls be used as an alternative to lead hazard abatement under certain circumstances; and
4. Establishes a commission to study current childhood lead poisoning prevention law, policies, and standards.

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To learn more about this project, please refer to the following sources:

The Dartmouth Community Outreach Core web page: http://www.dartmouth.edu/~toxmetal/OUmanch.shtml

New Hampshire SB 176 law page: http://www.gencourt.state.nh.us/legislation/2007/SB0176.html

Research Brief 152: Folic Acid Supplementation Lowers Blood Arsenic by Increasing Arsenic Methylation

Release Date: 08/01/2007

Background: Methylation (the addition of methyl groups) of ingested inorganic arsenic to methylarsonic (MMA) and dimethylarsinic acids (DMA) occurs by enzymatic reactions that transfer the methyl group from S-adenosyl-methionine (SAM) to arsenic. Methylation of arsenic facilitates its urinary elimination and is considered a detoxification process. Because folate compounds play an important role in regulation of SAM levels, folate may also influence arsenic methylation and toxicity.

Chronic arsenic exposure currently affects more than 100 million people worldwide.

People pumping water from a hand pumped well

The Columbia University SBRP is a collaboration of highly interactive research projects focused on improving our understanding of health effects, geochemistry, hydrology and remediation of arsenic in groundwater. Their aim is to lower the health risks associated with exposure to arsenic in groundwater, by guiding the focus of interventions in high-risk areas. The Columbia SBRP has established the Health Effects of Arsenic Longitudinal Study, a cohort study with a population of nearly 12,000 individuals exposed to a wide range of inorganic arsenic (InAs) from drinking water in Araihazar, Bangladesh. The Columbia group has also made methodological advances increasing our capabilities to measure total arsenic and arsenic metabolites in blood, where concentrations are an order of magnitude lower than those in urine. They found that blood arsenic is a biomarker of arsenic exposure and is directly associated with the risk for arsenic-induced skin lesions.

Dr. Mary Gamble is building upon this work to investigate nutritional influences on arsenic metabolism and toxicity. Her research team documented a high prevalence of hyperhomocysteinemia (abnormally high levels of homocysteine in the blood; caused by deficiencies in folic acid, B6 or B12) and folate deficiency among Bangladeshi adults; these conditions were both associated a reduced capacity to methylate arsenic.

Naturally occurring folates are highly susceptible to degradation during harvesting, storage, processing, preparation, and passage through the acidic environment of the stomach. Remarkably, up to 95% of naturally occurring food folates can be destroyed by prolonged cooking alone.

Advances: Image of Drs. Allan Rosenfield, Joseph Graziano and Mary Gamble discussing the progress of the columbia Program during a visit to Araihazar, BangladeshIn a recent, randomized, double-blind, placebo controlled trial of folic acid supplementation, Dr. Gamble and colleagues 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 in urine. 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 concentrations.

Her team tested this hypothesis by measuring total arsenic and arsenic metabolites in blood of 130 participants with low plasma folate before and after 12 weeks of supplementation with folic acid (400 µg/day, equivalent to the US Recommended Daily Allowance) or placebo. Folic acid supplementation lowered total blood arsenic concentrations by increasing the methylation of InAs and MMA to DMA, which is rapidly excreted in urine. This was evidenced by reductions in the concentrations of InAs and MMA in blood and a rapid increase in the concentration of DMA in urine. Specifically:

- 85% of all participants in the folic acid group experienced a decline in levels of MMA in blood (bMMA).
- Average bMMA reduction in the folic acid group was 22%, with greater declines observed for individuals who had higher bMMA at the beginning of the trial.
- There was no change in DMA in blood in the folic acid group after the 12 week intervention, because once DMA is formed, it is rapidly excreted in urine.
- Inorganic arsenic in blood decreased by nearly 19% for the folic acid group.
- Folic acid supplementation reduced total blood arsenic by 14%.

Significance: This 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. Dr. Gamble concludes that 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. This research, which provides information critical to the identification of potential targeted interventions for preventing arsenic toxicity, could have significant impacts on public health and policy.

Left to right: Drs. Allan Rosenfield, Joseph Graziano and Mary Gamble discussing the progress of the columbia Program during a visit to Araihazar, Bangladesh

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To learn more about this research, please refer to the following sources:

Gamble, Mary V., X. Liu, Vesna Slavkovich, J. Richard Pilsner, Vesna Ilievski, Pam Factor-Litvak, Diane Levy, S. Alam, Mohammad N. Islam, Faruque Parvez, Habibul Ahsan, and Joseph H. Graziano. 2007. Folic acid supplementation lowers blood arsenic. American Journal of Clinical Nutrition (http://www.ajcn.org/). (in press)

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Research Brief 153: Novel High-Strength Iron Cement Sequesters Pollutants

Release Date: 09/05/2007

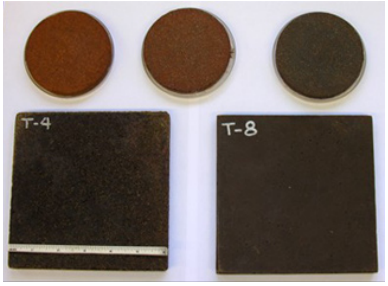
Background: Traditional industrial processes are being updated to consume less material, generate less waste, and use less energy. In addition, researchers and engineers are developing new methods to complement established processes. These secondary systems transform waste streams into feed stocks for new products that can break down or safely sequester toxic materials. These "industrial ecology" approaches seek to eliminate pollution by integrating recycling at all stages of a product's life cycle - from acquisition of raw materials to reuse of parts/materials when the product no longer functions.

Mr. David Stone, a graduate student working with University of Arizona SBRP researchers Joan Curry and Janick Artiola, is using solid waste materials from sources such as open pit copper mines, coal-fired power plants, steel foundries, cement plants, and shot blasting facilities to create hard aggregate materials for use in industrial and environmental applications.

Advances: Guided by processes in nature, Mr. Stone has developed a bench-top method to create an iron-based cement, which incorporates carbon dioxide during the curing process. The basic solid material components are zero-valent iron and silica. Mr. Stone uses fly ash as the predominant silica source because of its wide availability, and small, consistent particle size.

The largest and most widespread source of cheap iron is scrap steel, which must be reduced to powder - a process that requires energy input. The researchers use an electrochemical cell to dissolve the steel at the anode in an acid bath, which results in the deposition of zero-valent iron at the cathode. The brittle deposit is broken off of the cathode and crushed into granules. This process can be made both economically feasible and environmentally benign by using solar cells as the DC power source.

Preliminary studies indicate that iron prepared in this way forms very strong aggregate materials. The researchers mix the iron, silica, and other materials (promoters, catalysts, and stabilizers) and add water to form a paste that can be deposited in a mold or placed directly and troweled smooth, just like Portland cement products. The aggregate material hardens upon exposure to carbon dioxide, which is absorbed and permanently trapped within the mineral matrix. A 5/8" thick, 12"x12" tile, which weighs 5 kg, can sequester about 1 kg of CO_2. The iron carbonate composite falls into the category of "super-strong" cements. The material can have greater strength than Portland cement, and functions more effectively under certain extreme conditions such as those found in seawater and other corrosive environments. On-going work is focused on improving the process, investigating the material micro-structure and the chemistry of the curing process.



Examples of iron carbonate cement. Compositional differences produce slight variations in color. The 6-inch scale bar on the lower left sample indicates the dimensions of the tiles and disks.

The University of Arizona has filed a patent application to protect this discovery.

Significance: Metallic iron is a common filtrate for a variety of environmental contaminants including the dense non-aqueous phase liquids trichloroethylene and perchloroethylene; metals such as arsenic, cadmium, chromium, copper, lead, and zinc; and radioactive materials. The iron-based cement production process developed at the University of Arizona could be used to bind these toxic

materials in a cement matrix. The SBRP researchers have plans to test the material's ability to permanently sequester metals as well as organics. As such, this research effort has great potential to protect human and environmental health.

Image of UA SBRP Investigator Joan Curry and David Stone at the 2007 University of Arizona Technology Innovation Award Luncheon

The researchers believe that their process is particularly intriguing because it not only consumes waste materials, but also consumes a primary green house gas. This is of great interest to the Portland cement industry because carbon dioxide is a major and unavoidable by-product of cement production. It is possible that carbon dioxide emissions from Portland cement plants could be used as an input stream for the production of an iron-carbonate waste-based cement material. This could lead to savings (or profits) as it could: reduce costs of processing industrial waste materials; yield carbon credits for sequestering CO_2 during the manufacturing process; and produce a marketable product that could be used as building panels, tiles, block, pavers, etc.

For his discovery of this material, Mr. Stone received the 2007 University of Arizona Student Technology Innovation Award.

Update (October 01, 2007): David Stone, Joan Curry and Janick Artiola have been awarded a $50,000 supplemental grant from the SBRP to continue testing the iron-based composite material for the stabilization of industrial wastes, and potentially, arsenic bearing residuals. This supplemental grant will allow for ongoing work to improve the process, investigate the material s micro-structure and the chemistry of the curing process.

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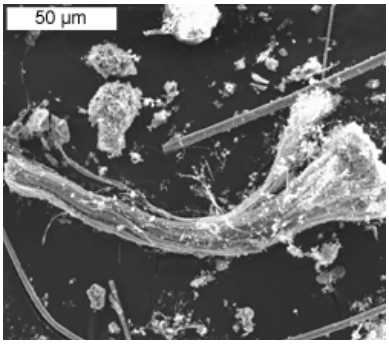
Research Brief 154: Understanding the Underlying Mechanism of Asbestos Carcinogenesis

Release Date: 10/03/2007



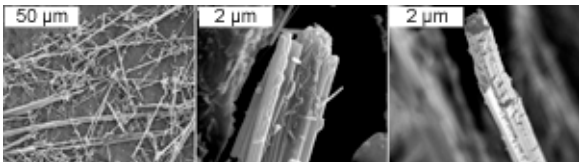
Chrysotile bundle, World Trade Center dust

Background: Asbestos fibers, one of the most significant environmental hazards in the world, are established carcinogens for humans and animals. Both experimental and epidemiologic data have shown that asbestos exposure is closely associated with the development of pulmonary fibrosis, bronchogenic carcinoma, and malignant mesotheliomas of the pleura and peritoneum. Although occupational exposure is considered the main route of exposure, there is evidence that asbestos-associated diseases are linked to family members of asbestos workers and in residents living near industrial sources of asbestos. The finding in 2000 that nearly one third of the 4,500 residents of Libby, Montana, some as young as ten years old, had developed asbestos-related diseases from exposure to tremolites-contaminated vermiculites, exemplifies the human toll of environmental asbestos exposure. This highlights the significance of chronic, non-occupational asbestos exposures via their homes, schools, neighborhoods, or naturally occurring geologic sources.

It has been reported that fiber dimension, bio-persistence, composition and surface reactivity are important criteria for the carcinogenicity of the fibers, indicating that carcinogenic mechanisms of asbestos are likely to be complex and involve multiple pathways. Previous work established that asbestos is an effective gene and chromosomal mutagen and that the mutagenicity is mediated by reactive oxygen species (ROS). However, the mechanisms by which asbestos produces malignancy in vivo are unclear.

To gain a better understanding of the mechanism by which asbestos induces malignancy, researchers at the Columbia University SBRP lead by Dr. Tom K. Hei performed two studies using chrysotile and crocidolite asbestos fibers.

Advances: In a study building on Dr. Hei's previous findings that asbestos is mutagenic and induces multilocus deletions in mammalian cells, the research team used embryo fibroblast cells from the gpt delta transgenic mice to quantify and characterize the deletions induced by graded doses of chrysotile fibers.

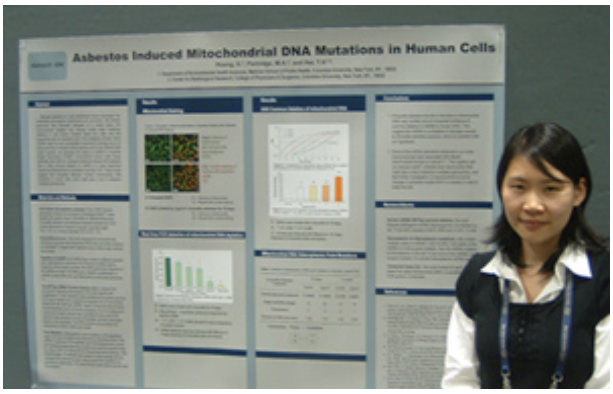


Crocidolite asbestos fibers (UICC standard)

They found that exposure to varying doses of chrysotiles for 24 hours produced a *dose-dependent decrease in cell viability*. Chrysotiles also induced a *dose dependent increase in the number of mutations*. Both the incidence and types of mutants resulting from exposure to chrysotile fibers were comparable to those induced by equitoxic doses of hydrogen peroxide, (H_2O_2), a powerful oxidant that not only induces damage to DNA, causing single- and double-strand breaks, base loss, base substitution, and cross-linking, but also causes chromosome and chromatid aberrations.

In an effort to understand the molecular mechanisms involved in the intra-chromosomal deletions induced by chrysotile, the researchers compared mutation patterns between chrysotile asbestos and ROS. The results showed that for chrysotile-induced mutants, the ratios of mutants with large deletions were similar to those induced by H_2O_2 at equitoxic doses. This suggests similar mutagenic mechanisms for asbestos fibers and ROS.
The study also showed that damage resulting from treatments with chrysotile fibers was inhibited by concurrent treatment with catalase. These findings provided strong corroborating evidence of the DNA damaging effects of chrysotiles through the oxyradical pathway.

To determine if nucleus is the necessary and sufficient target for asbestos-induced DNA damages, Dr. Hei's research team then performed a series of analyses using cytoplasts (without nucleus) exposed to crocidolite fibers to determine if mutations in the DNA can still be scored upon fusion of crocidolite asbestos-exposed cytoplasts with nucleus from untreated cells.



Ph.D. student Sarah Huang at the ACRR meeting in Los Angeles, 2007

The study demonstrated that the nucleus need not be present at the time of fiber treatment to initiate the genotoxic events. Furthermore, crocidolite treatment of enucleated cytoplasts initiated oxyradical generation in a dose-dependent manner. Both the oxyradical generation and the mutation yield in the fused cells were dramatically decreased by concurrent treatment with dimethyl sulfoxide (DMSO), an effective radical scavenger.

The study also found that crocidolite fibers induced a dose-dependent increase of nitric oxide (NO) production, which was suppressed significantly with NG-methyl-L-arginine (L-NMMA), a nitric oxide synthase inhibitor. Similarly, there was a dose-dependent decrease in the mutation yield induced by crocidolites in the presence of graded doses of L-NMMA. Using western blotting for nitrotyrosine coupled with fluorescent probes, the investigators further concluded the importance of peroxynitrite anions in mediating asbestos induced DNA damage.

Significance: Although asbestos is a well-established human carcinogen, the underlying carcinogenic mechanisms are not clear. That asbestos has been used so extensively in industry and its physical durability make it an important health concern. Although the U.S. EPA has restricted the industrial use of asbestos in the U.S. since the early 1970's, the continued discovery of routes through which the general public may be exposed to asbestos suggests a long-term, low dosage exposure of a large number of people. Furthermore, there is evidence to indicate that the epidemic of asbestos-related diseases is rapidly emerging in developing countries where the magnitude of human toll is significantly higher than in the U.S. and Western Europe. A better understanding of the genotoxic/carcinogenic mechanisms of this omnipresent environmental carcinogen is the only sure way to design effective interventional measures. The present studies from the SBRP laboratory at Columbia University provide evidence on the role of reactive oxygen and nitrogen species in the DNA damaging effects of asbestos and that extranuclear target could be important in the process. The research findings provide a basis for improved therapies for asbestos-related diseases.

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To learn more about this research, please refer to the following sources:

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Research Brief 155: Assessing Bioremediation of Chloroethenes through Stable Carbon Isotope Fractionation

Release Date: 11/07/2007

Background: Chloroethenes, such as tetrachloroethene (PCE), trichloroethene (TCE), isomers of dichloroethene (DCE), and vinyl chloride (VC), are among the most prevalent and problematic groundwater pollutants. These compounds pose serious health threats due to their toxicity and potential carcinogenicity. *In situ* bioremediation is often used at contaminated sites, but it is difficult to quantify the effectiveness of the biological processes because of changes that can result from physical processes, such as dissolution, volatilization, and sorption.

Dr. Lisa Alvarez-Cohen at the University of California, Berkeley SBRP believes that quantification of shifts in stable carbon isotope ratios is a promising approach for evaluating the effectiveness of in situ bioremediation systems. Physical processes cause minor or negligible shifts in isotope ratios, while biological conversion of organic compounds can cause significant changes in the ratio of carbon isotopes (^{13}C : ^{12}C) in both the reactants and products. In biological transformations, reaction rates tend to be faster for molecules with light isotopes than for molecules with heavy isotopes. As a result, the residual reactant becomes enriched with the heavy isotopes and the daughter products are enriched with the light isotopes. This enrichment (fractionation) can be measured using compound-specific stable isotope analysis.

In situ bioremediation of TCE can occur by microbial reductive dehalogenation. The process involves the sequential reduction of TCE to DCE, followed by conversion of DCE to VC (a known human carcinogen), and finally VC to the innocuous end product ethene.

Advances: Early fractionation studies performed with mixed bacterial cultures from field samples yielded highly variable isotopic fractionation effects. This suggests that different organisms utilizing different pathways to degrade chloroethene compounds may cause different isotopic shifts.

Dr. Alvarez-Cohen's research group studied stable carbon isotope fractionation during the reduction of TCE using three test systems representing phylogenetically distinct organisms:

- Pure cultures of bacterial strains that are known to dechlorinate chloroethenes completely to ethene (*Dehalococcoides ethenogene*s 195 and *Dehalococcoides* sp. strain BAV1)
- A well-characterized mixed culture containing *Dehalococcoides*
- Pure isolates of bacterial strains that dechlorinate TCE to DCE, but do not complete the metabolism through VC to ethene (*Sulfurospirillum multivorans* and *Dehalobacter restrictus* strain PER-K23)

This is the first measurement of carbon isotope fractionation by *Dehalococcoides* isolates and of TCE transformation by *S. multivorans* and *D. restrictus* strain PER-K23. The extent of fractionation varied widely, yet within each culture constant enrichment factors were observed. Dechlorinating cultures exhibited a range of enrichment factors at each dechlorination step, and strains within the same genus or species generated significantly different enrichment factors. Additionally, isotope fractionation generated by a microbial community was quite different from that generated by isolates. These results offer an interesting comparison and insight into factors that affect biological fractionation.

The large range of TCE enrichment factors observed, coupled with the reported similarities of the reductive dehalogenase enzymes (RDases), suggest that these biological fractionations are governed by a combination of the structure of the native enzyme and its cofactor, transport, and enzyme-

substrate binding prior to the carbon-chlorine bond-breaking step. As a result of these findings, Dr. Alvarez-Cohen believes that general categorization of enrichment factors without specific measurements can result in misinterpretation and that caution should be exercised in selecting appropriate values for quantitative analysis to predict the extent of dechlorination.

Significance: Compound-specific stable isotope analysis is a valuable tool for assessing *in situ* bioremediation at contaminated sites. However, as a result of wide variation in enrichment factors measured for both bacterial isolates and enriched communities, this study found that it is important to estimate site representative factors to optimize estimates of degradation rates.

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To learn more about this research, please refer to the following sources:

Lee, Patrick K H, Mark E. Conrad, and Lisa Alvarez-Cohen. 2007. Stable carbon isotope fractionation of chloroethenes by dehalorespiring isolates. Environmental Science and Technology (http://pubs.acs.org/journals/esthag/). 41(12):4277-85.

Research Brief 156: Enhanced Phytoremediation of Volatile Environmental Pollutants

Release Date: 12/05/2007

Background: There is a great deal of interest in the use of trees to clean up contaminants found at hazardous waste sites. As a result of natural processes fueled by solar energy, trees offer great potential for environmentally sound and cost-effective clean-up of contaminated soil, groundwater, and air. Some tree species, particularly poplars, grow at a remarkable rate - up to 15 feet per year - and their root systems penetrate large volumes of soil. In addition to drawing water and nutrients out of the ground, these trees also take up organic contaminants and metabolize them to harmless products. It is well-documented that poplar trees metabolize trichloroethylene (TCE) to innocuous products. There is no release or sequestration of toxic intermediate metabolites such vinyl chloride, a known carcinogen.

This remediation strategy - known as phytoremediation - has many advantages over engineering methods such as "pump and treat", including being significantly less expensive, less intrusive, and more aesthetically pleasing. The disadvantages of phytoremediation are that it is often too slow, may be only seasonally effective or removes only small amounts of pollutant from the environment. Regulatory agencies often require significant progress in remediation to be demonstrated in only a few years, making most phytoremediation applications unsuitable.

Image of chamber with plant growing inside

Advances: Since the 1980's, the NIEHS Superfund Basic Research Program has funded cutting-edge phytoremediation research at the University of Washington. Initiated by the late Dr. Milton Gordon, this work has evolved from identification of plant species best suited to remediation, to development of more effective hybrids, to on-going studies to develop a transgenic poplar (*Populus tremula x Populus alba*) with greatly increased rates of metabolism and removal of volatile hydrocarbons including TCE, vinyl chloride, carbon tetrachloride, benzene, and chloroform.

In a paper published in the October 23, 2007 Proceedings of the National Academy of Sciences, Drs. Stuart Strand and Sharon Doty report on their work to transform hybrid poplar with a vector containing rabbit cytochrome P450 2E1. The resulting plants overexpress cytochrome P450 2E1, a key enzyme in the metabolism of a variety of halogenated compounds.

In laboratory studies that exposed apical stem cuttings to TCE, the scientists found that CYP2E1-containing transgenic cuttings had average rates of TCE metabolism nearly 45-fold greater than in the control cuttings. Two of the CYP2E1 transgenic lines had TCE metabolism rates that were more than 100-fold higher than in controls. The transgenic cuttings grew normally and did not display any adverse reaction to the TCE or its metabolites.

Dr. Doty working in the lab

Drs. Strand and Doty then conducted studies to determine if the increased metabolism of TCE in the poplar cuttings led to increased uptake from solution and/or from air. They found that while control cuttings removed <3% of the TCE from hydroponic solution, the CYP2E1 transgenics removed 51% to 91%. When exposed to contaminants in the air, the transgenic plants also demonstrated increased uptake. The CYP2E1 plants removed 79% of the TCE from the air, while control plants did not show any statistically significant uptake of TCE. In 2 weeks, the control plants removed an average of only 13% of benzene from air, while two CYP2E1 plant lines removed 36% and 46%.

Significance: The SBRP researchers believe that this work represents the first development of transgenic trees for increased removal of a broad range of serious environmental pollutants from water and air. Additional studies are needed to verify efficacy under field conditions and to ensure that plant tissues do not cause unacceptable impacts on non-target organisms.

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To learn more about this research, please refer to the following sources:

Strand, Stuart E., Sharon Lafferty Doty, Jasmine Wilkerson, and Federico M. Farin. 2007. Enhanced phytoremediation of volatile environmental pollutants with transgenic trees. Proceedings of the National Academy of Sciences of the United States of America (PNAS) (http://www.pnas.org/). 104(43):16816-16821.

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