2014 SUPERFUND RESEARCH PROGRAM *Annual Meeting*

POSTER ABSTRACTS

UC BERKELEY SUPERFUND RESEARCH PROGRAM
SCIENCE FOR A SAFER WORLD

NIH National Institute of Environmental Health Sciences
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TECHNICAL ABSTRACT

Identifying N-6 Adenine-Specific DNA Methyltransferase 1 (N6AMT1) polymorphisms associated with arsenic methylation using a custom and low-cost genotyping method in two population studies

We previously demonstrated that N-6 adenine-specific DNA methyltransferase 1 (N6AMT1) is involved in methylation of the toxic inorganic arsenic (iAs) metabolite, monomethylarsonous acid (MMA), to the less toxic dimethylarsonic acid (DMA) in human urothelial cells. Therefore, polymorphisms in the N6AMT1 gene may play a role in susceptibility to arsenic toxicity by altering methylation of MMA to DMA. A recent study by Harari et al. found associations between five tag single nucleotide polymorphisms (tag SNPs) located within the N6AMT1 gene and the percentage of MMA in the urine of arsenic-exposed and unexposed people. Building on these findings, we identified 14 tag SNPs that provide broader coverage of the N6AMT1 gene and designed a custom multiplexed, ligation-dependent probe amplification (MLPA) assay to genotype these tag SNPs. MLPA is an inexpensive and rapid genotyping method. Urinary metabolites (%iAs, %MMA, %DMA) and N6AMT1 tag SNP genotypes were measured in an arsenic-exposed population from Cordoba, Argentina (n=141). An initial analysis identified a statistically significant association between tag SNP rs1003671 genotype (GG) and both %MMA and %DMA in urine. No relationships were observed between the remaining tag SNPs and urinary arsenic metabolites or between tag SNPs and cancer case status. A larger analysis was performed on an arsenic-exposed population from Northern Chile comparing urinary metabolites and N6AMT1 tag SNPs (n=601). No significant associations were identified between tag SNPs or haplotypes and urinary arsenic metabolites or cancer case status. These preliminary results, from two different populations, reveal that human N6AMT1 polymorphisms alone might not predict susceptibility to arsenic toxicity.

LAY ABSTRACT

Identifying genetic differences that contribute to vulnerability to arsenic toxicity

Arsenic is a toxic chemical that is found at high levels in water in some parts of the world. We previously established the involvement of a gene that protects humans by converting arsenic from a more toxic form to a less toxic one. Therefore, differences in the DNA sequence of this gene could alter its function and make a person more or less efficient at converting ingested arsenic to its less toxic form. Furthermore, less efficient converters might be more vulnerable to the adverse health effects of arsenic exposure such as bladder, kidney, and lung cancers. A recent study found that arsenic-exposed individuals with certain DNA sequence changes in this gene had a higher percentage of the more toxic arsenic form in their urine. Building on these findings, we selected a larger number of DNA sequence changes to more precisely explain the relationship between these changes and gene function. An initial analysis on an arsenic-exposed population from Cordoba, Argentina found that one DNA sequence change was associated with a decrease in the more toxic arsenic form in urine. No relationships were observed between all other selected DNA sequence changes and urinary arsenic forms or between DNA sequence changes and cancer case status. A larger analysis was performed on an arsenic-exposed population from Northern Chile comparing urinary arsenic and DNA sequence changes. No significant associations were identified between DNA sequence changes, urinary arsenic forms, or cancer case status. These preliminary results reveal that this gene alone might not predict vulnerability to arsenic toxicity.

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TECHNICAL ABSTRACT

Electrochemical dechlorination of TCE in the presence of natural organic matter, metal ions and nitrates in a simulated karst aquifer

A small-scale flow-through limestone column was used to evaluate the effect of common coexisting organic and inorganic compounds on the dechlorination of trichloroethylene. This system consists of an iron anode and a copper foam cathode. In the absence of humic acid (organic matters) and dichromate, selenate, and nitrate (inorganic matters), 90% of initial TCE was dechlorinated under optimized conditions (90 mA current, 1 mL/min flow rate, and 1 mg/L initial TCE concentration). Humic acid out-competes for the reactive sites on iron anode with TCE. Its aggregates inhibit the reduction rate of TCE to some extent. Metal ions compete with TCE for electron transformation, as they are strong oxidants. The added hexavalent chromium was completely reduced to trivalent chromium due to the ferrous species from iron anode. Chromium precipitates could cover the iron anode surface and prevent further anode reactions. With dichromate, TCE reduction rate decreased around 1.5 times. Selenate is less effective than dichromate on TCE remediation as the removal efficacy of TCE decreased around 10%. Selenate complexes with dissolved iron result in aggregates, which may also coat the iron surface and reduce dechlorination rate. Although the system is not efficient to treat nitrate as well as metal ions, the present investigations indicate that the electrochemical reduction on a copper foam cathode is capable of significantly remediating TCE (around 80%), even in the presence of a high concentration of nitrate (40 mg/L). This system can be engineered and optimized to treat TCE co-contamination with a relatively wide variety of contaminants.

LAY ABSTRACT

Removal of trichloroethylene in the presence of natural organic matter, metal ions and nitrates by an electrochemical process

A lab-scale column was used to evaluate the effect of common coexisting organic and inorganic compounds on the chlorine removal from a toxic substance, such as trichloroethylene (TCE). This process is called dechlorination. There are some different pathways leading to dechlorination. We are using electrochemical process that consists of an iron anode and a copper foam cathode. Both humic acids (organic matters), which are the major constituents of soil and water and metal ions (inorganic matters), which are strong oxidants, compete with TCE in electron transformation process. To determine this, various concentrations of humic acids, dichromate, selenate, and nitrate added to the solution containing TCE prior to running the experiments. In the absence of humic acid (organic matters) and dichromate, selenate, and nitrate (inorganic matters), 90% of initial TCE was removed under optimized conditions (90 mA current, 1 mL/min flow rate, and 1 mg/L initial TCE concentration). The added hexavalent chromium was completely reduced to trivalent chromium. With dichromate, TCE removal efficiency decreased around 1.5 times. Selenate is less effective than dichromate on TCE remediation, as the removal efficacy of TCE decreased around 10%. Although the system is not efficient enough to remove nitrate, the present investigations indicate that the electrochemical dechlorination on a copper foam cathode is capable to significantly remove TCE (around 80%), even in the presence of a high concentrations of nitrate (40 mg/L). This system can be engineered and optimized to treat TCE co-contamination with a relatively wide variety of contaminants.

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TECHNICAL ABSTRACT

Development of a dual estrogen receptor recombinant cell line for detection of estrogenic/antiestrogenic chemicals

Alterations in reproduction and endocrine homeostasis, reductions in sperm counts, and dramatic increases in human breast and prostate cancers are responses likely resulting from environmental contaminants adversely affecting hormone action. Endocrine disrupting chemicals (EDCs) have been identified in a wide range of environmental, biological, commercial and consumer products and food, and many EDCs exert their effects through mimicking/inhibiting the action of estrogen on its receptors. We previously developed a recombinant human ovarian carcinoma cell line (BG1Luc4E2) that has received regulatory approval by the USEPA and OECD as an accepted bioassay for detection of estrogenic/antiestrogenic chemicals. This bioassay is suboptimal for screening since the BG1 cell line contains only one of the two known estrogen receptor (ER) subtypes, ER alpha. The ability of the two ER subtypes (ER alpha/ER beta) to be activated/inhibited by similar and different chemicals indicates that the BG1Luc4E2 bioassay only detects a subset of all estrogenic/antiestrogenic chemicals. Accordingly, we reengineered the BG1Luc4E cell line so it now expresses both ER subtypes and thus can detect the full range of estrogenic/antiestrogenic chemicals. The utility of the new cell line was demonstrated through the use of ER beta selective ligands and the rapid detection of estrogenic chemicals present in a chemical library of common insecticides, herbicides, industrial and household chemicals. (NIEHS SRP ES004699)

LAY ABSTRACT

Development of a dual estrogen receptor recombinant cell line for detection of estrogenic/antiestrogenic chemicals

Pollution still poses one of the biggest threats to ecosystems and human health. Assessing pollution damage is difficult because the adverse effects observed in humans and animals in these areas typically result from combined exposure many different toxic chemicals. Endocrine disruptors represent a relatively new class of contaminant that affects hormone-dependent biological processes, such as reproduction. Endocrine disruptors can be released into the environment from a wide variety of natural and human-derived materials and given their potential for adverse effects, there is a critical need to be able to easily detect these chemicals in environmental, biological and food samples. Accordingly our laboratory developed a recombinant human cell bioassay (BG1Luc4E2) that could be used for detection of estrogenic chemicals in sample extracts based on their ability to activate the estrogen receptor. While our previous assay was useful, these cells lack the other known estrogen receptor and thus can't detect the full range of actual estrogenic chemicals. Accordingly, we reengineered the recombinant BG1Luc4E2 cell line such that they now express both forms of the estrogen receptor and these new cells respond to the full range of estrogenic chemicals. The utility of this new recombinant cell line demonstrated by the rapid screening of a chemical library containing pesticides, herbicides, industrial and household chemicals for estrogenic activity. (NIEHS SRP ES004699)

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TECHNICAL ABSTRACT

Detection of Biomarkers of Inflammation in newborns using filter paper blood sampes from metabolic screening

While traditional risk factors for preterm births (PTBs) like a lack of prenatal care, maternal education and access to health care services have improved in Puerto Rico (PR), the island maintains the highest rate of PTBs among all United States jurisdictions. PR also has the highest density per square mile of Superfund Sites. These sites contain toxicants such as phthalates, chlorinated solvents, and pesticides. Therefore, it is important to investigate whether potential exposure to contaminants contributes to the high prevalence of PTBs in PR. PROTECT is conducting targeted and non-targeted analysis to develop a centralized data repository to understand cumulative risk factors and develop prevention strategies for PTB. Since environmental exposure to contaminants like phthalates has been identified as a possible trigger of inflammation responses, and inflammation is one of the pathways that may pose a risk for preterm births, this project aims to establish a method to detect biomarkers of inflammation in newborns using readily available blood samples from filter paper of newborn metabolic screening. To achieve this, we propose the development of an assay capable of measuring biomarkers for inflammation in newborns. This assay would use mass spectrometry methodology and equipment used by the newborn screening program to detect inflammation markers such as IL-6. These measurements, coupled with the environmental exposures data also collected through PROTECT, would allow for the comparison of cases that present increased markers of inflammation with those that do not, thus contributing to the discovery of more specific biomarkers for preterm risk.

LAY ABSTRACT

Detection of markers of Inflammation in newborns

While traditional risk factors for preterm births (PTBs) like a lack of prenatal care, maternal education and access to health care services have improved in Puerto Rico (PR), the island maintains the highest rate of PTBs among all United States jurisdictions. PR also has the highest density per square mile of Superfund Sites. These sites contain toxicants like pesticides. Therefore, it is important to investigate whether potential exposure to contaminants contributes to the high prevalence of PTBs in PR. PROTECT is conducting specific analysis to develop a database to understand risk factors and develop prevention strategies for PTB. Since environmental exposure to contaminants has been identified as a possible trigger of inflammation responses, and inflammation is one of the pathways that may pose a risk for preterm births, this project aims to establish a method to detect markers of inflammation in newborns using readily available blood samples taken and stored as part of routine newborn screening. To achieve this, we propose the development of a test capable of measuring markers for inflammation in newborns. This test would use methodology and equipment used by the newborn screening program to detect inflammation markers. These measurements, coupled with the environmental exposures data also collected through PROTECT, would allow for the comparison of cases that present increased markers of inflammation with those that do not, thus contributing to the discovery of more specific markers for preterm risk.

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TECHNICAL ABSTRACT

Iron Mediated Electrochemical Activation of Persulfate: Fate of Electrons and Oxidants

Persulfate (S2O82-) is an increasingly used oxidant for the treatment of many recalcitrant contaminants. Because of its slow reaction kinetics with organics, S2O82- can be activated into SO4●- to accelerate the rate of contaminant oxidation, but most activation approaches are impractical for small-scale treatment systems. Previous research has demonstrated rapid activation of S2O82- by dissolved Fe(II) to produce SO4●- and oxidized Fe(III). Slow reduction kinetics of Fe(III) back to Fe(II), however, makes metal catalyzed activation impractical. To assess the potential for using electrochemistry to enhance the rate at which Fe(II) is regenerated, Fe(III)-citrate was reduced on a cathode in the presence of S2O82- and a representative organic contaminant (phenol). In the absence of Fe(III), persulfate was effectively activated by direct electron transfer on the cathode but most of the SO4●- was further reduced on the cathode. Upon addition of Fe(III)-citrate, phenol underwent rapid disappearance with rates following first order kinetics. Maximum phenol removal was observed for a current density of 5 A m-2 at a concentration of 10 mM persulfate and Fe-citrate and 1mM phenol. At lower current densities, the reaction kinetics were limited by the applied current, while at higher currents mass transfer limitations reduced the efficiency of the process. Approximately 20% of the activated persulfate reacted with phenol oxidation under optimal conditions. Electrochemical activation of persulfate with ferric citrate serving as a catalyst may be a practical alternative to advanced oxidation processes for treatment of hazardous and industrial waste streams that contain dissolved iron or iron-complexing ligands.

LAY ABSTRACT

Electrochemical Treatment of Pollutants using Persulfate

The presence of trace concentrations of industrial pollutants in wastewater streams (e.g., pesticides, solvents, pharmaceuticals) have raised concerns due to their environmental persistence and toxicity. Persulfate (S2O82-) is an increasingly used chemical for the treatment of many of these recalcitrant contaminants. Due to its slow reaction speed with contaminants, it is typically activated into an even more reactive compound (the sulfate radical) by metals such as iron as well as by heating the water. Many of these persulfate activation techniques, however, are energy intensive, time consuming, and generate waste compounds that make them impractical for small-scale treatment systems. Using electrochemistry in conjunction with iron for persulfate activation is an effective alternative because the rate of treatment can be adjusted easily and reactants can be conserved. The goal of this research is to provide insight into the potential for using electrochemistry to enhance contaminant removal by persulfate and to find a niche application of where this technology could be implemented. We tested several different solution conditions and studied how quickly and efficiently a model contaminant (phenol) was degraded. Our results show that the electrochemical activation of persulfate with iron may be a practical alternative to existing persulfate activation technologies for the treatment of hazardous waste streams already containing dissolved iron such as those from industrial steel manufacturing.

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TECHNICAL ABSTRACT

Development of polyphenolic nanocomposite materials for rapid removal of organic pollutants from contaminated water sources

LAY ABSTRACT

Development of novel materials for rapid removal of organic pollutants from contaminated water sources

Novel magnetic nanocomposites were developed for the capture of organic pollutants from water sources. Two different synthesis methods were used to develop magnetic iron oxide nanoparticle based composites functionalized with polyphenolic compounds expected to have high affinities for organic pollutants. The first approach utilized a core-shell composite where iron oxide nanoparticles were coated with a polymer containing polyphenols using a surface initiated polymerization. The second approach was based on a polyphenol-containing polymer matrix with dispersed magnetic nanoparticles that was ground into micron size particles (magnetic nanocomposite microparticles or MNMs). Both platforms allow for the specific binding of chlorinated organics, the magnetic separation of bound organics from contaminated water sources, and the thermal destabilization of the polymer matrix for contaminant release and material regeneration. The polyphenols, which are naturally occuring nutrients, are expected to have high affinities for chlorinated organics and are theorized to form binding pockets in the polymer structure that mimic antibody binding sites in the human body. These polyphenols were acrylated forms of naturally occurring curcumin (curcumin diacrylate or CDA), and Quercetin (quercetin multiacryate or QMA). The physical properties and composition of these innovative materials were characterized with commonly used techniques (TEM, SEM, DLS, FTIR, TGA, XRD, etc.). Additionally, pollutant binding studies were conducted using polychlorinated biphenyls (PCBs, specifically PCB 126) as a model organic pollutant so as to determine binding affinity and capacity. It was demonstrated that both novel materials effectively bound PCBs, and the addition of the polyphenolic compounds resulted in greater affinity.

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TECHNICAL ABSTRACT

Oxidative Treatment of Poly- and Perfluoroalkyl Compounds in Aqueous Film Forming Foams

Aqueous film-forming-foams (AFFFs) have been used for several decades by the military, oil refineries, airports, and municipal firefighters to extinguish petroleum-based fires. Numerous polyfluorinated chemicals are present in AFFF formulations, including perfluorinated carboxylates and sulfonates, as well as compounds known to be precursors to the perfluorinated carboxylates and sulfonates. To assess the fate of poly- and perfluorinated compounds in AFFF during oxidative chemical treatment and to develop in situ chemical oxidation (ISCO) methods for these compounds, time course experiments were performed using dilute solutions of perfluorooctanoic acid or one of two AFFF formulations. Solutions were amended with Fenton's reagent, activated persulfate, permanganate, or a combination of persulfate and permanganate. Persulfate treatment resulted in transformation of PFOA to shorter-chain PFCA products, while treatment with Fenton's reagent and permanganate did not. The degree of PFOA transformation by persulfate was less in systems containing synthetic groundwater or aquifer sediments as compared to systems prepared in deionized water. The apparent transformation of PFOA by Fenton's reagent is likely due to sorption, not reaction. In AFFF manufactured by the Ansul Company, near complete transformation of 6:2 fluorotelomer thioamido sulfonate was observed for all treatments. Treatment of Ansul AFFF by Fenton's reagent or persulfate resulted in transformation of n:2 fluorotelomer thioamido sulfonates to n:2 fluorotelomer sulfonates, which were subsequently transformed to perfluorinated carboxylates of equal or lesser fluorocarbon chain length. Permanganate treatment of Ansul AFFF produced only small amounts of fluorotelomer sulfonate and perfluorinated carboxylic acid transformation products.

LAY ABSTRACT

Chemical Treatment of Fluorinated Pollutants in Groundwater

Numerous classes of industrial pollutants have been detected in groundwater, and the number of known of pollutants is constantly increasing. Each pollutant has a unique chemical structure that affects how long the pollutant persists in the environment, how fast and how far it is likely to travel, and how toxic it is to humans and other organisms. Perfluorinated compounds (PFCs) are a class of chemicals that have received increasing attention due to their persistence, their toxicity, and their presence in water resources around the world. These compounds contain a large number of fluorine atoms, which make PFCs both remarkably useful as non-stick coatings and particularly difficult to degrade. PFCs were widely used as a component of firefighting foams, and recently they have been detected in groundwater at firefighter training sites where these foams were used. Currently, the standard way of remediating these sites is to pump the groundwater to the surface and run the water through a carbon filter. While this is an effective way of removing PFCs from water, it is time consuming, energy intensive, and in the end, the PFC-containing carbon must be disposed of as hazardous waste. The goal of this research is to develop chemical treatments that could be injected into groundwater to destroy PFC contamination in place. We tested several different chemical treatments and studied whether and how quickly different PFCs were degraded. Our results show that degrading PFCs to harmless byproducts using chemical treatment may be more difficult than some scientists initially believed.

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TECHNICAL ABSTRACT

A highly sensitive fluorescence enzyme immunoassay based on nanobody-alkaline phosphatase fusion protein for monitoring food contaminant ochratoxin A

Most of the previously reported immunoassays for OTA are developed with monoclonal antibodies or polyclonal antibodies, and are carried out with primary or secondary antibodies which were chemically labeled with enzymes, such as horseradish peroxidase. However, it has been reported that the chemical conjugation of enzymes to antibodies presented some drawbacks, such as the low stability and safety of the reagents used for labeling. With the rapid development of antibody engineering, single chain fragment of variable (scFv)-alkaline phosphatase (AP) fusions is considered an attractive alternative for simple and rapid immunoassay analysis, avoiding the chemical conjugation of enzymes to antibodies and the use of a second antibody. However, scFvs are often observed to have poor solubility and stability which require additional genetic engineering to improve. As an alternative to conventional antibody, nanobody (Nb) which is derived from the variable region of heavy chain antibody existing in camelids and sharks, is more attractive than scFv. Nb (15 kDa) is smaller than scFv (75 kDa) and exhibits high solubility and the ability to refold properly after denaturation by heat or chemical means. In our previous work, we reported on the panning of binders specific for OTA from an immunized alpaca Nb library, and sixteen binders with four different amino acid sequences were selected. In this study, one binder with the highest sensitivity in phage ELISA was used to prepare the Nb-AP construct for expression. The purified fusion protein was used to develop a sensitive fluorescence enzyme immunoassay (FEIA) for OTA detection in cereal.

LAY ABSTRACT

One-step direct competitive fluorescence enzyme immunoassay for detection of ochratoxin A in cereal

OTA is a secondary metabolite primarily produced by Aspergillus and Pencillium species, which can contaminate cereal and cereal products around the world. Many studies have revealed the diverse toxicities of OTA, including teratogenic, mutagenic, carcinogenic, hepatotoxic, immunosuppressive, and nephrotoxic effects. ELISA is routinely used to screen large amounts of food samples for monitoring OTA because of its high sensitivity, easy operation and high-throughout analysis. In most sandwich ELISA formats, the recognition antibody is either itself conjugated with an enzyme or followed by a secondary antibody that has been conjugated with an enzyme that catalyzes signal generation, most commonly color formation. Alternatively, protocols have been developed in which the primary and secondary antibodies are added to the assay in one step. This is also true when using Nbs as the detection antibody, which requires the use of a secondary antibody such as an anti-Histidine tag antibody chemically conjugated to an enzyme such as HRP or AP. Advances in recombinant DNA technology have allowed production of antibody-AP fusions, which can eliminate the use of chemically produced enzyme-antibody conjugates and decrease ELISA analysis time. In this work, the Nb-AP fusion protein was constructed and used to develop a fast and sensitive one-step FEIA for OTA. Validation results from FEIA and LC-MS/MS were in good agreement with each other, indicating the reliability of this assay for application in the rapid screening of OTA in cereal.

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TECHNICAL ABSTRACT

Effects of ferrous and ferric iron on dechlorination kinetics of trichloroethene to ethene by Dehalococcoides mccartyi

Predicting the performance of in situ bioremediation requires an understanding of geochemical effects on microorganisms in dechlorinating communities. The effects of ferric and ferrous iron on dehalorespiration of trichloroethene (TCE) and its daughter-products, dichloroethene (DCE) and vinyl chloride (VC), to ethene by Dehalococcoides mccartyi 195 were observed in pure culture. Amorphous Fe(II) and Fe(III) in 1 mg/L and 10 mg/L concentrations were amended to pure D. mccartyi 195 cultures with 77 µmol TCE, 5mM acetate, and vitamins, including 100 µg/L cobalamin. No significant changes in dechlorination kinetics were observed in cultures amended with ferric iron. Inhibition of VC reduction was observed in cultures amended with 10 mg/L ferrous iron. In contrast to the culture without ferrous exposure, VC dechlorination was inhibited by 20% at day 30. By day 50, the culture with 10 mg/L ferrous exposure reached 88% completion of VC dechlorination, whereas all other cultures had reduced at least 92% of VC to ethene by day 30. QPCR analysis indicated that concentrations of 1 mg/L ferric and ferrous iron had no effect and a slight effect on cell growth, respectively (no amended iron, 2.49x10^7 cells/mL; 1 mg/L ferric iron, 2.53x10^7 cells/mL; and 1 mg/L ferrous iron 1.27x10^7 cells/mL). However, both iron forms inhibited cell growth at 10 mg/L (ferric iron 2.42x10^6 cells/mL; ferrous iron 2.65x10^6 cells/mL). This information improves our understanding of potential geochemical inhibitions of D. mccartyi and can help us to develop mechanisms for alleviating stresses caused by iron on the dechlorination activities of D. mccartyi 195.

LAY ABSTRACT

Effects of common soil components on the biological transformation of trichloroethene to ethene

Hundreds of millions of people in the United States rely on groundwater as their drinking water source, making groundwater contaminants like trichloroethene (TCE) a serious threat to human health. To achieve successful groundwater contaminant removal, it is important to understand how soil components affect the microorganisms at contaminated sites. We studied the effects of ferric and ferrous iron (common soil components) on the transformation of TCE to non-toxic ethene by the microorganism Dehalococcoides mccartyi 195. We found that higher concentrations of ferrous iron slowed the transformation of vinyl chloride (a chemical formed as an intermediate step of this process) to the desired end product, ethene, while no other conditions showed significant changes in the speed of contaminant transformation. Additionally, we found that higher concentrations of both ferric and ferrous iron significantly diminished the growth of the microorganisms, and low concentrations of ferrous iron had a slight, measurable effect on growth. This information improves our understanding of the effects of common soil components on the TCE transformation process, and can help us to develop methods for relieving the stresses caused by iron on the biological TCE remediation process.

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TECHNICAL ABSTRACT

The Role of Endogenous and Environmental AhR ligands in Mammary Epithelial Cell Tumor Growth and Invasion.

Historically, AhR activation by environmental ligands was seen to facilitate mutations through CYP1 up-regulation and mutagen production. Our findings suggested that AhR activity drives cancer in the absence of environmental ligands without mutation. Our long-term goal is to identify endogenous ligands present in human mammary tumor cells, to determine how their production is controlled, and to assess how environmental AhR ligands alter this process. We hypothesize that metabolites produced by the kynurenine (KYN) pathway of tryptophan metabolism drive AhR activity and enforce invasion of breast cancer cells. As a corollary, we predict that environmental AhR ligands distort this signaling. We found that the tryptophan metabolites kynurenine (KYN) and xanthurenic acid (XA), both detected in Hs578T cell lysates, are potent AhR agonists. Hs578T cells highly express TDO, the rate-limiting enzyme in the kynurenine pathway. TDO knock-down reduced Cyp1B1, a transcriptional target of the AhR, as well as the expression of invasion-related genes MMP1 and 9, cell migration in a wound healing assay, and invasive growth in Matrigel. We also found that AhR activity contributes to transcriptional regulation of TDO. Our results support the hypothesis that AhR, hyper-activated by endogenous ligands produced via the KYN pathway, contributes to the malignant phenotype in breast cancer. Moreover, our finding that the AhR transcriptionally regulates TDO expression leads us to propose that environmental AhR ligands lead to increased production of endogenous AhR ligands by up-regulating TDO in a positive feed-back loop and thereby enforcing AhR-driven cell malignancy.

LAY ABSTRACT

The Role of an Environmental Pollutant Sensor, the Aryl Hydrocarbon Receptor (AhR), in Driving Breast Cancer via Disrupted Amino Acid Production.

This study focuses on a cell protein referred to as the aryl hydrocarbon receptor (AhR), an environmental chemical sensor long associated with cancer. The AhR is activated by ubiquitous chemicals found in grilled food, cigarette smoke and car exhaust. AhR-activating chemicals are also formed during industrial processes such as paper bleaching and waste incineration. Historically, AhR activation by these environmental pollutants was thought to lead to production of DNA-damaging products, resulting in DNA mutations and the initiation of several types of cancer including breast cancer. However, recent evidence suggests that molecules produced by the cells themselves also can hyper-activate the AhR in cancer cells and that this hyper-active AhR contributes to advanced stages of breast cancer including lethal metastasis. Our goal is to determine what cell-derived molecules activate the AhR and how production of these molecules is influenced by environmental pollutants. Our findings from studies with human breast cancer cells suggest that metabolism of an amino acid, tryptophan, leads to products capable of engaging and activating the AhR on an on-going basis resulting in increased tumor aggressiveness. We also found that activated AhR can influence production of enzymes involved in production of the above-mentioned tryptophan-derived products in a self-sustaining positive feed-back loop. Understanding the molecular mechanisms through which the AhR, and by implication environmental pollutants, lead to agressiveness of tumors will allow us to develop evidence-based precautionary measures relevant to the harmful effects of environmental pollutants in cancer patients and will open new avenues for therapeutics and diagnostics.

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TECHNICAL ABSTRACT

Arsenic uptake and accumulation in rice.

Rice cultivars that restrict arsenic accumulation in the grain offers one of the simplest, fastest and most cost effective approaches to solving the problem of arsenic contamination of rice and rice-based products. We have been screening a large collection of rice cultivars and have identified several that have grain arsenic concentrations that place them in the tails of the distribution observed. Using genetic markers spaced across the genome, we have recently identified associations between markers on the short arm of chromosome 11 and variation in grain As. In addition to following arsenic concentration in grains, we are also using synchrotron X-ray fluorescence mapping to localize arsenic as well as essential macro- and micronutrient elements (K, Ca, Mn, Fe, Ni, Cu and Zn) in developing grains of rice exposed to arsenic through the cut flag leaf. Grains were exposed to either 25 or 100 mg/L arsenate (AsV), or 25 mg/L dimethylarsinic acid (DMA) during grain development (expressed as days post anthesis). When exposed to 100 mg/L AsV, arsenic localized in both the embryo and the endosperm, whereas at 25 mg/L AsV exposure, arsenic accumulated in the ovular vascular trace. We inferred that the efficiency of DMA transport into the endosperm was greater than AsV at all tested concentrations. Grains exposed to AsV later in grain development had a higher maximum As abundance, suggesting that arsenic transport activity increases as the grain matures. Localization of As in the grain is a product of both species and exposure concentration.

LAY ABSTRACT

Arsenic uptake and accumulation in rice.

Rice – the seed of the grass Oryza sativa – is an important staple of the human diet. Rice takes up arsenic from the soil, causing concerns for human health, particularly in young children; those who eat a lot of rice, and those already exposed to arsenic through drinking water. The amount of arsenic that reaches the edible parts of the rice grain varies from cultivar to cultivar. Finding rice cultivars that do not accumulate arsenic in the grain offers the simplest, fastest and cheapest way to protect human health. We have screened a large collection of rice cultivars and have found those that accumulate different amounts of arsenic. These cultivars will allow us to find the genes responsible for moving arsenic into the grain. We have collected images of arsenic in the rice grain after exposure to different arsenic species (arsenate and dimethylarsinic acid, or DMA), concentrations and stages of grain development. This tells us to what extent these factors influence where arsenic accumulates in the rice grain. The presence of arsenic in distinct parts of the grain tells us which rice-based products may pose a greater risk, and shows us where arsenic transport proteins operate. Our work addresses the long term challenge of developing arsenic-free rice and the short term challenge of helping consumers understand which rice-based foods may contain arsenic.

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TECHNICAL ABSTRACT

Degradation of Polychlorinated Biphenyls Driven By Light-Activated Multicomponent Nanomaterials

Polychlorinated biphenyls (PCBs) are notorious pollutants that represent a significant threat to environmental and human health. Although the production and use of PCBs has been banned in many countries for more than 30 years, their toxic effects still remain from their release into the environment. An effective method to reduce the environmental burden of PCBs is to design efficient reductive dechlorination-based systems for remediation of these toxic species. In order to achieve this goal using sustainable chemical processes, we have demonstrated that light-activated metal oxide materials decorated with noble metal nanoparticles can be used as highly efficient and green dechlorination systems for PCBs. For this, Pd nanoparticles were deposited onto Cu2O cubes to generate a composite structure. The photocatalytic activity of the Cu2O/Pd materials was studied via the dechlorination of monochlorinated PCBs 1, 2, and 3 and PCB 77, a model tetrachlorinated specimen. Our data indicate that the rate of dechlorination is affected by the location and number of chlorine substitutions and that removal of chlorines is favored in order of para > meta > ortho positions. Our work is likely to affect environmental mitigation technologies and thus provide sustainable, transformative approaches for in-field degradation methods.

LAY ABSTRACT

Degradation of Polychlorinated Biphenyls Driven By Light-Activated Multicomponent Nanomaterials

Chlorinated molecules are well known pollutants with toxic effects on the environment and human population. Although their use has been banned in many countries for more than 30 years, PCBs were released into the environment before they were banned, thus their toxic effects are still a great problem. An effective method to reduce this problem is to design efficient degradation systems that are easy to use and do not require energy. Thus, new catalysts, or materials that speed up chemical reactions without changing their structure, are required for long-term destruction of PCBs in the environment. We hypothesize that sunlight can be used as a renewable energy source to breakdown PCBs. For this, we have designed and developed small catalytic particles that showed degradation of multiple PCB molecules, with different chemical structures. The data suggest that the speed of degradation is affected by the chlorine atom in the PCB. This research could be used for the production of new materials that use sunlight for toxicant destruction, opening up great possibilities for their widespread use.

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TECHNICAL ABSTRACT

Evaluating Bacterial and Archaeal Community Structure and their Potential for in situ Bioremediation of Polycyclic Aromatic Hydrocarbons

Polyaromatic hydrocarbons (PAHs) are some of the most common industrial pollutants and exist in high concentrations at many Superfund sites. PAHs are difficult to remediate due to their stability and ubiquity, and limited natural remediation strategies exist. The Norfolk Naval Shipyard on the Elizabeth River, a Superfund site in Northern Virginia, is heavily contaminated with PAHs from decades of industrial development. The Superfund site has, along with other contaminants, large volumes of coal-tar creosote. Coal-tar creosote is highly toxic and carcinogenic and consists almost entirely of aromatic hydrocarbons. Further, the site's estuarine bacterial populations have additional natural stressors including salinity, temperature, and fluctuating water levels that require quick adaptations, making these communities ideal for modeling contamination impacts. Fifty-milliliter samples were collected at three sediment sites of varying contamination along the Elizabeth River. The PAH distributions were characterized using LC/MS-MS and the microbial communities are being compared using next generation sequencing methods. In particular, site community analysis provides information with regards to sensitive as well as resistant microorganisms. In the long term, we are interested in determining adaptations within the archaeal and bacterial populations. Finally, we hope to identify potential donor strains for the implementation of genetic bioaugmentation strategies.

LAY ABSTRACT

Determining Microbial Communities Profiles and Degradation Potential at the Elizabeth River Superfund Site

Contaminated water systems are a threat to environmental and human health. A contaminant of major concern are the polycyclic aromatic hydrocarbons (PAHs), a class of persistent organic compounds known to be carcinogenic (cause cancer). These hydrocarbons are some of the most common industrial pollutants and they are difficult to remediate due to how stable they are chemically. As a result, limited strategies exist to cleanup sites contaminated with PAHs. Heavily contaminated estuaries and creeks, which are also subject to stressors including water level and temperature, force adaptations (like genetic mutations or increased gene expression) within the microbial communities. As a result, estuarine bacterial populations are ideal models for adaptations and remediation. The Norfolk Naval Shipyard on the Elizabeth River, a Superfund site in Northern Virginia, serves as our model site. The site is heavily contaminated with PAHs from decades of industrial development, mostly coal-tar creosote from a wood processing facility. Coal-tar creosote is toxic and carcinogenic, as well as foul smelling. Using samples collected along the Elizabeth River, at varying contamination concentrations, we aim to investigate the specific effects of the pollution and the remediation. We are interested in discovering the short-term and long-term effects on the microbial communities, the effects of the contamination downstream, and possible ways to mitigate the damages. Our hypothesis is by modeling the bacterial communities adaption or development at the highly impacted site, we can develop an improved method to naturally and sustainably remediate contaminated sites.

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TECHNICAL ABSTRACT

Characterizing Fate and Transport of TCE Solvents in a Karst Groundwater System

Dense Nonaqueous-Phase Liquids (DNAPLs) are a group of organic compounds that have been a serious problem for groundwater pollution in karst. The physic-chemical properties of DNAPLs in conjunction with the hydraulic properties of the karst systems create the perfect condition for DNAPLs to penetrate the epikarst, reach the groundwater, and move within the karst system to zones of potential exposure, such as wells, streams and wetlands. This research studies the fate, transport, and distribution of trichloroethene (TCE) NAPL and associated dissolved species in kasrt groundwater systems. Transport experiments are carried in a karstified limestone physical model (KLPM), a limestone block enclosed in stainless steel tank and simulating a saturated confined karst aquifer. After injection of pure TCE solvent into a steady groundwater flow field, samples are taken spatially and temporally and analyzed volumetrically and with HPLC. Data show pure TCE volumes are collected at the beginnings of the experiment in sampling ports located near the injection port and along preferential flow paths. Results from the constructed temporal distributions curves at different spatial locations show spatial variations related to the limestone block heterogeneity. Rapid response to TCE concentrations is associated with preferential flow paths. Slow response with long tailing is indicative of diffusive transport in the rock matrix and mass transport rates limitations. Overall, results show that karstified limestone has a high capacity to rapidly transport, as well as store and slowly release TCE pure and dissolved phase.

LAY ABSTRACT

Mobility, Persistence, and Distribution of TCE in Karst Groundwater

Our interest is to develop fundamental knowledge in the processes controlling mobility, persistence, and distribution of trichloroethylene (TCE) solvents through karst groundwater system. TCE is a volatile organic carcinogen compound used commonly as degreaser, refrigerant, and components of adhesives, paints, and pesticides. The wide use and properties of TCE result in its widespread movement into the subsurface, groundwater, and zones of potential exposure such as wells, springs, streams, and wetlands. Karst groundwater systems, which are characterized by conduit and high permeability zones, may pose a higher risk of exposure. This research studies the fate and transport of TCE solvent in a kastified limestone physical model. The model consists of a limestone block enclosed and represents a saturated confined karst aquifer. TCE experiments involve the injection of pure TCE into steady groundwater flow, while sampling at different times along the block. The obtained data is used to constructed concentration distribution curves at different times and spatial locations. Flow and transport in the karst model occur through conduits and the pore matrix of the rock. Results show zones of preferential path flow where the solvent and dissolved components move rapidly to sample ports that are associated with the conduit features. Transport in the rock pore matrix occurs through diffuse flow and rate-limited mass transfer mechanisms. These zones serve as long-term storage reservoirs that can slowly release TCE over long periods of time.

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TECHNICAL ABSTRACT

Microbial Recovery of Te0 Nanoparticles from Tellurate (TeO4 2-)

There is a great need for cost-effective and selective recycling methods to ensure a sustainable supply of scarce, critical elements needed for advanced technology. Biotechnology offers great promise to concentrate and refine these elements from waste streams. This research focuses on the microbial recovery of tellurium (Te0) from aqueous streams containing soluble tellurium species. Tellurium is a critical metalloid with a broad range of applications ranging from photovoltaic cells, medical applications, and additive to enhance optoelectronic and thermal properties of steel and glass.
The aim of this work was to investigate the recovery of Te0 nanoparticles from the soluble oxyanion tellurate (TeO4 2-) using an anaerobic mixed culture. Two electron donors, acetate and H2, were evaluated for their effectiveness in promoting the reduction of (TeO42-). The effect of four redox mediators (anthraquinone-2,6-disulfonate (AQDS), hydroxocobalamin, riboflavin and lawsone) was also tested. The results obtained in batch anaerobic bioassays revealed that Te reduction occurred only in the presence of microorganisms. The rate of Te reduction was enhanced in the presence of H2 and it was remarkably accelerated when lawsone was used as the redox mediator. The formation of Te0 nanoparticles was confirmed by electron microscopy analysis (TEM EDS). These encouraging results indicate that the use of redox mediators can accelerate microbial formation of Te0. Future research will consider the recovery of Te0 in a continuous-flow anaerobic bioreactor.

LAY ABSTRACT

Recovery of Tellurium using a Microbial Process

Elemental tellurium (Te0) is a metalloid utilized in numerous industrial processes, including manufacture of items such as solar panels, optical blu-ray disks, and steel. Tellurium is as scarce as gold and silver, and growing demand for this critical element has led to an urgent need for effective recycling methods to ensure its continuous supply. Tellurium is also a toxic chemical; thus, effective recovery of tellurium will also help minimize potential environmental damage. Biotechnological methods offer promise for recovering tellurium from wastes, wastewater and leachates. Some microorganisms are able to transform tellurate, a soluble form of tellurium, into insoluble Te0 that can be easily recovered from the original solution utilizing conventional methods like filtration.
In this work, the conversion of soluble tellurium (tellurate or TeO42-) to solid Te0 using microorganisms was studied. Two food sources which act as electron donors, acetate and hydrogen gas (H2), were evaluated for their effectiveness in promoting the formation of Te0 in the presence or absence of the microorganisms. The ability of four chemical compounds to accelerate Te0 production by affecting electron transfer was also tested. The results obtained revealed that the formation of Te0 was a biological process that only occurred when microorganisms were present. The rate of Te0 formation was enhanced in the presence of hydrogen and it was remarkably accelerated (seven times) by one of the chemical compounds. These encouraging results indicate the potential of biotechnological methods to recover tellurium.

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TECHNICAL ABSTRACT

One-step Immunoassay Using Camelid Single Domain Antibody-Alkaline Phosphatase Fusion Protein to Detect Tetrabromobisphenol A

Tetrabromobisphenol A (TBBPA) is a ubiquitous brominated flame retardant, showing widespread environment and human exposures. Naturally occurring antibodies in camelids (termed as VHH) that are devoid of light chain, is the smallest sized functional antigen-binding entities. Ease of genetic manipulation makes VHHs a superior choice in the development of immunoreagents. In this study, a highly selective VHH for TBBPA fused with alkaline phosphatase (AP) from Escherichia coli, integrated TBBPA-binding with enzymatic detection. The one-step immunoassay was developed in 5% dimethyl sulfoxide (DMSO)/phosphate buffered saline (pH 7.4), with the limit of detection (LOD) and the inhibition half-maximum concentration (IC50) for tetrabromobisphenol A of 0.03 and 0.20 ng mL−1, respectively. Negligible cross reactivity (<0.1%) of the assay was observed with a set of important brominated analogs. It appears the C15-AP fusion protein had larger paratope capacity for heterologous TBBPA coating antigens due to dimerization of AP. Although characteristic of C15-AP is greatly influenced by temperature, pH value and organic solvents, higher sensitivities and specificities were observed in C15-AP-based assay. Diluting urine samples directly in DMSO is a unique sample preparation technique that resulted in recoveries of TBBPA ranging from 96.7 % to 109.9 %. Dimerized VHHs show promise in contaminant detection.

LAY ABSTRACT

A Novel One-Step Assay to Measure the Flame Retardant Tetrabromobisphenol A

A one-step immunoassay was developed to measure the environmental contaminant tetrabromobisphenol A (TBBPA), which is the most widely used of brominated flame retardants. Immunoassays use antibodies to capture or bind to the TBBPA. Most antibodies have a molecular size of 150 kilodaltons. Our study used a smaller antibody (only 15 kilodaltons) that is uniquely found in camelids. Unlike most antibodies, these smaller antibodies (VHH) are more water soluble, do not degrade under high temperature and can be easily manipulated using molecular biology techniques yet bind as well as their larger counterparts. Using genetic techniques we fused an enzyme called alkaline phosphatase (AP) to the VHH that binds to TBBPA. This fusion protein (C15-AP), allowed us to develop a quick assay that could measure TBBPA as low as 0.03 ng mL−1. The assay did not detect other brominated flame retardants. The assay using C15-AP was not as robust as the assay that used the C15 alone. However, the C15-AP fusion protein bound differently than the C15 alone probably due to the nature of the AP protein. We are currently studying this novel phenomenon. To test the utility of the assay we added known amounts of TBBPA to urine samples. We were able to recover a range of 96.7 % to 109.9 % of the amount that was added. We conclude that the VHH genetic fusion with AP is an effective method to detect TBBPA in human samples.

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TECHNICAL ABSTRACT

Correlating Fungal Presence and Community Structure to Contaminant Profile at the Atlantic Wood Industries Superfund Site

Remediation of heavy polycyclic aromatic hydrocarbons (PAHs) is challenging due to their inherent recalcitrance in soil. Heavy PAHs have physicochemical properties leading to strong sorption to soil particles and low solubility in water. Thus, remediation approaches are often limited to in situ stabilization and excavation-neither of which directly transforms the contaminants. More sustainable techniques such as traditional bacterial bioremediation find limited success due to the large size and low availability of heavy PAHs. However, fungi have been well documented to degrade many PAHs via nonspecific extracellular enzymes. This work examines the native fungal diversity across a gradient of contamination to identify PAH impact on fungal communities. To achieve this objective, total genomic DNA was extracted and characteristic fungal gene regions were amplified. Then, amplicons were sequenced in a high throughput manner using the Illumina sequencing platform. Quantitative Insights into Molecular Ecology (QIIME) was paired with R for statistical analyses and transformation of raw sequences. Finally, the Classifier tool developed at Michigan State's Ribosomal Database Project was used to assign taxonomy. PAH concentration profiles were obtained using gas chromatography coupled with electron impact mass spectrometry (GC/EIMS). Resulting correlations between fungal occurrence and PAH profile may reveal a fungus or fungal community that is well-suited to degrade PAHs. Preliminary results suggest a decrease in diversity between clean and contaminated soils. They also suggest that the communities are mainly dominated by ascomycete fungi, with members among the basidiomyota and zygomycota. These results will shape future fungal bioremediation strategies to address PAH soil contamination.

LAY ABSTRACT

Linking Contaminants to Fungal Presence and Diversity at the Atlantic Wood Industries Superfund Site

Cleaning soils that are contaminated with creosote is difficult largely because of the strength with which it attaches to the soils. Creosote is a mixture of more than 30 different polycyclic aromatic hydrocarbons (PAHs)-some mutagenic and carcinogenic. Our main sustainable remediation approach involves bacteria that use contaminants as food. They consume the contaminants and transform them into more innocuous compounds as part of their metabolism. This approach is not well suited to creosote soil contamination because heavy PAHs often associate too closely with soils to be available to bacteria and are too large to cross into their cells. In contrast, fungi release enzymes directly into their environment and then use degradation products to grow. They can change the contaminant outside their cells. That metabolic difference makes them less limited by contaminant availability than most bacteria. Therefore, they are good candidates for improving PAH soil contamination remedies. Here, we look into the native fungi tolerating different PAHs using a technique that identifies all fungi present in a given soil. If useful fungi are already in the soil, we do not necessarily need to introduce different ones. We have identified preliminary trends and aim to see if further studies reveal a particular fungal community well-suited to PAH remediation. Preliminary results show decreasing diversity with increasing PAH concentration. These results also reveal that the communities are mainly composed of sac fungi, but have a few representatives within the basidiomycota and zygomycota. We will incorporate these results in a strategy for PAH remediation.

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TECHNICAL ABSTRACT

Misregulation of OPT3 Results in Tissue Specific Changes in Cadmium Accumulation

Plants are a major source of essential dietary metals such as zinc, iron, and manganese, but are also the main point of exposure to toxic heavy metals and metalloids including cadmium, lead, and arsenic. We found that the Arabidopsis oligonucleotide transporter mutant, opt3-2, causes a shift in the tissue specific sequestration of cadmium, resulting in an over-accumulation of cadmium in the seeds and roots but an under-accumulation in the leaves. In addition, the constitutive upregulation of iron uptake transporter IRT1 and iron reductase FRO2 in roots of the opt3-2 mutants show a constitutive iron-deficiency response. Leaf-specific expression of OPT3 can complement both the cadmium sensitivity and the IRT1 expression in roots. OPT3 expression was found primarily in the plasma membrane of the phloem and appears to play a key role in phloem-loading of iron and tissue distribution of metals. We are continuing this research with the goal of changing the tissue-specific distribution of heavy metals in order to minimize transport of toxic heavy metals to the aerial portions of the plant. This root targeted sequestration of toxic heavy metals will be extremely beneficial in crops, such as apples, lettuce and rice, that are known to have high toxic heavy metal concentrations in aerial, edible tissues. We are currently working to use root-specific promoters to drive heavy metal detoxification machinery, we have previously identified, in order to trap toxic heavy metals in the roots and minimize loading into the vasculature.

Mendoza-Cózatl D.G., et al. (2014). Mol. Plant. 00, 1–16.

LAY ABSTRACT

Plants are a major source of essential dietary metals such as zinc, iron, and manganese, but are also the major point of exposure to toxic heavy metals and metalloids including cadmium, lead, and arsenic. We found that in the Arabidopsis mutant line, opt3-2, there is a shift in the tissue specific storage of cadmium, resulting in increased accumulation of cadmium in the seeds and roots but decreased accumulation in the leaves. In addition, we find increased activity of iron uptake and processing proteins (IRT1 and FRO2) in the roots of these mutants, which is associated with an iron-deficiency response. Leaf-specific expression of OPT3 can counteract the changes in both cadmium accumulation and IRT1 expression. OPT3 expression was found primarily in the plasma membrane of the phloem and appears to play a key role in phloem-loading of iron and tissue distribution of metals in plants. We are continuing forward with the goal of changing the tissue-specific distribution of heavy metals in order to minimize transport of toxic heavy metals to the aerial portions of the plant, such as seeds, fruits and leaves. This root targeted storage of toxic heavy metals will be extremely beneficial in crops, such as apples, lettuce and rice, that are known to have high toxic heavy metal concentrations in aerial, edible tissues. We are currently working to use root-specific promoters to drive heavy metal detoxification machinery, we have previously identified, in order to trap toxic heavy metals in the roots and minimize loading into the vasculature.

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TECHNICAL ABSTRACT

Structural analysis of Dehalococcoides mccartyi within a TCE-dechlorinating community maintained in a steady-state Chemostat

A 3.0 liter completely mixed flow reactor (designated CANAS) with a 75 mL d-1 flow rate was established and steady-state was achieved during the experiment (200 days). At steady-state, 1.7 mM TCE was converted to ethene 1.50± 0.10 mM, vinyl chloride 0.09± 0.06 mM and cis-DCE 0.03 ± 0.01 mM. Two distinct D. mccartyi strains ANAS1 and ANAS2 were stably maintained at 6.2 ± 2.8×108 cells mL-1 and 5.8 ± 1.2×108 cells mL-1, respectively. Electron balance analysis during steady-state operation demonstrated 104.4% electron recovery, in which 8.4 % of the electrons consumed went to dechlorination while 77.4% were stored in propionate and acetate, 11.8% electrons contributed to biomass production and 2.4% were stored as methane and H2 in the reactor. Metagenomic analysis revealed that the community structure of CANAS shifted significantly from the original long-term ANAS inoculum culture (semi-fed-batch mode). The abundance of Desulfovibrio and Methanobacterium species decreased significantly, while Clostridium and Pseudomonas were enriched in CANAS. Genomes of these four species were recovered from metagenomic datasets and genomic analysis revealed that TCE reductive dechlorination genes were missing in all the genomes, suggesting a lack of dechlorination capacity in these microorganisms. D. mccartyi was the dominant species in both the chemostat and semi-batch conditions, and reductive dechlorination was not affected by the shift in community structure. A genus-wide microarray was applied to study the transcription level of D.mccartyi species in CANAS under steady-state conditions. The transcription analysis identified tceA and vcrA to be among the most expressed genes in CANAS.

LAY ABSTRACT

Structural analysis of a microbial community that could detoxify TCE in a completely mixed flow reactor

In order to understand the function and behavior of a complex microbial community in bioremediation sites and further to predict the detoxification performance during bioremediation, we need a better understanding of the microbial abundance, distribution, dynamics, and functions in a continuous flow system that are more representative of contaminated plumes. In this study, we obtained a completely mixed flow reactor (designated CANAS) that was maintained at steady-state (all metabolites concentrations were stable), and the reactor could efficiently detoxify over 88% of TCE (target contaminant) to the benign end product ethene, with other intermediate metabolites maintained in the reactor. The microorganisms that were responsible for TCE detoxification were maintained at stable levels during the experiment. Electron balance analysis during steady-state operation demonstrated 8.4 % of the electrons (stored in the form of lactate) consumed went to TCE detoxification, while 77.4% were stored in the form of organic acids, 11.8% electrons contributed to biomass production and 2.4% were stored as methane and hydrogen in the reactor. Metagenomic analysis revealed that the community structure of CANAS shifted significantly from the original long-term ANAS inoculum culture (semi-fed-batch mode). For those microorganisms that significantly increased/decreased, genomes information were recovered from metagenomic datasets and genomic analysis revealed that there was a lack of detoxification capacity in those microorganisms. However TCE detoxification performance was not affected by the shift in microbial community structure. This information, along with gene expression studies, could help us to identify the key microorganisms involved in bioremediation processes at different growth conditions.

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TECHNICAL ABSTRACT

LAY ABSTRACT

Comparing different methods of measuring bioavailability during sediment remediation

There are many places in the world where ocean sediments have been contaminated by persistent organic pollutants (POPs). Often this comes from historic manufacturing activities and use, such as the contamination of DDT in the Palos Verdes Shelf in Southern California. There are different strategies that allow for the cleanup, or remediation, of these sites, an example being black carbon amendments (i.e., the addition and mixing of activated carbon to the sediment). The goal for amending the sediment is to reduce the bioavailability of POPs. An effective amendment will decrease the bioavailability by causing these POPs to become adsorbed or immobilized and keep the POPs from being taken up by organisms. We have used three methods to determine the bioavailability of DDT and DDE in sediments after black carbon amendment: solid-phase microextraction (SPME), Tenax desorption, and isotope dilution method (IDM). We tested charcoal, activated carbon, and sand by mixing them into the sediment at various ratios. Compared to the unamended sediments, all three methods were able to predict essentially the same reductions in bioavailability for the various amendment treatments. This finding suggests that different methods of determining the bioavailability of POPs may be used to determine the remediation effectiveness of contaminated sediments. However, two methods, (Tenax and IDM), were consistently more sensitive in detecting reductions in bioavailability than SPME. This information may be used for selecting bioavailability measurement methods when evaluating remediation effectiveness and progress.

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TECHNICAL ABSTRACT

Removal of 1,2,3-trichloropropane in groundwater by granular activated carbon

1,2,3-trichloropropane (1,2,3-TCP) is a carcinogenic pesticide impurity and solvent/degreaser. It was detected in 251 active public wells in California from 2002 to 2012. 1,2,3-TCP is resistant to many conventional treatment methods and has a groundwater half-life of 1-2 years. Granular activated carbon adsorption is an effective treatment method, but meeting the proposed public health goal of 0.7 ng/L will be costly, necessitating treatment optimization.
Five granular activated carbons were compared for 1,2,3-TCP removal via batch kinetic experiments, isotherms, and two rapid small scale column tests (RSSCTs). The RSSCT breakthrough curves were compared to Homogeneous Surface Diffusion Model (HSDM) predictions at small and projected full-scale operation. The results indicated the importance of natural organic matter to adsorption capacity. The single solute HSDM predicted much longer operation times prior to breakthrough compared to the experimental data. In the RSSCTs, one groundwater had four times higher organic carbon content than the other, which corresponded to 35-77% earlier breakthrough for the water with higher organic matter. The performance of the carbons, from best to worst, was: F400 > CMR Lincave > Aquacarb 1230CX > OLC12x40 > OLC12x30.

LAY ABSTRACT

Removal of 1,2,3-trichloropropane in groundwater by granular activated carbon

1,2,3-trichloropropane (1,2,3-TCP) is a cancer-causing contaminant that was detected in 251 groundwater wells in California from 2002 to 2012. It commonly occurs as a byproduct of the pesticide 1,3-dichloropropene and seeps into groundwater during pesticide application. It degrades very slowly in natural systems and is difficult to remove by many water treatment methods. This research compared the abilities of five different granular activated carbons to remove 1,2,3-TCP from groundwater. The first experiment consisted of contaminated groundwater flowing through small-scale fixed carbon beds. In the second experiment, activated carbon was placed in constant-volume batches of contaminated water. The concentration of 1,2,3-TCP at the outlet of the small columns was monitored over time to compare how long each carbon could remove 1,2,3-TCP from the water before its removal ability started to become exhausted, causing the outlet concentration of 1,2,3-TCP to increase. The experimental outlet concentration data was compared to a mathematical model. The experimental results showed that the activated carbon removed significant amounts of organic matter in the groundwater, which reduced the amount of 1,2,3-TCP it could remove. The mathematical model did not factor in organic matter and predicted that the carbon would last much longer than was observed in the experiment. The performance of the carbons, from best to worst, was: F400 > CMR Lincave > Aquacarb 1230CX > OLC12x40 > OLC12x30.

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TECHNICAL ABSTRACT

LAY ABSTRACT

Effect of Metal Cluster Size on Environmentally Persistent Free Radical Formation and Toxicity

We know that radical species are usually highly reactive and therefore short-lived. The unusual nature of the newly discovered pollutants, Environmentally Persistent Free Radicals (EPFRs) are long-lived (for weeks and months) radicals found in the solid by-products (particulate matter, fly ash) of combustion systems. EPFRs are formed through a series of reactions between a metal-oxide (i.e. Cu, Fe, Ni) and an organic precursor (i.e. chlorophenols and chlorobenzenes). EPFRs exhibit extreme stability in the environment and biological systems, including our bodies. When we breathe in these particulates, they get deposited on our lungs and cause respiratory and cardiovascular problems.

The study presented here focuses on the effect of the metal cluster size relationship with subsequent radical formation. All radical characterization was done using Electron Paramagnetic Resonance (EPR) Spectroscopy. Copper is a well-known metal oxide that reacts to form EPFRs and is present in most fly ashes. We used seven different concentrations (0.25-5%) to look at how the change in cluster size affects radical species, lifetime and yield. The copper oxide samples were exposed to three organic precursors. Thus far, we have found that there are differences in the radical species formed between the precursors and within the seven concentrations for a single precursor. We noticed that at smaller cluster sizes a more stable radical was formed indicated by a change in the type of radical species formed.

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TECHNICAL ABSTRACT

Screening Nonionic Surfactants for Enhanced Biodegradation of Polycyclic Aromatic Hydrocarbons in Contaminated Soil

Effective bioremediation of soil contaminated with polycyclic aromatic hydrocarbons (PAHs) may be limited by the fractions of PAHs that are less bioavailable to PAH-degrading microorganisms. We evaluated the effects of adding surfactants on removal of the PAHs remaining after laboratory-scale, aerobic biological treatment of PAH-contaminated soil from a former manufactured-gas plant site. Five nonionic surfactants (Brij 30, Span 20, Ecosurf™ EH-3, polyoxyethylene sorbitol hexaoleate [POESH], and a rhamnolipid biosurfactant) were evaluated for their ability to enhance PAH desorption and biodegradation in the bioreactor-treated soil at two doses less than that required to reach the critical micelle concentration in the aqueous phase. The effects of surfactant-amended treatment on soil cytotoxicity and genotoxicity were also evaluated for Brij 30, Span 20, and POESH using the chicken DT40 B-lymphocyte cell line and two of its isogenic DNA-repair-deficient mutants.

Incubation of the bioreactor-treated soil with all surfactants resulted in modest increases in PAH desorption as measured with an infinite-sink desorption method. All surfactants except the biosurfactant substantially increased PAH biodegradation in the bioreactor-treated soil relative to the no-surfactant control. POESH had the greatest effect, resulting in removal of 52% of total measured PAH. Brij 30, Span 20, and POESH were particularly effective at enhancing biodegradation of four- and five-ring PAH, with removals up to 80%. All treatments except POESH at the optimum dose for PAH removal significantly increased soil cytotoxicity. Only the no-surfactant control and Brij 30 at the optimum dose significantly decreased soil genotoxicity as evaluated with either mutant cell line.

LAY ABSTRACT

Using Surfactants to Improve the Bioremediation of Soil Contaminated with Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds that occur frequently at contaminated industrial sites; many are acutely toxic and some are considered to be human carcinogens. Bioremediation, which relies on the activity of microbes to degrade pollutants, is one option for the treatment of PAH-contaminated soil. PAHs, however, are often strongly bound to soil and might be unavailable to degrading microorganisms. The application of surfactants has been proposed as a means of stimulating the release of PAHs from soil in order to increase biodegradation. In this study, five non-ionic surfactants were evaluated for their ability to enhance PAH release and biodegradation in contaminated soil that had already undergone conventional bioremediation in a bioreactor.

Treatment of the soil with all surfactants resulted in modest increases in the amount of PAHs released from the soil. Four out of 5 surfactants increased PAH biodegradation relative to further treatment without surfactant. The most effective surfactants significantly enhanced the biodegradation of 5 of the 7 PAHs considered probable human carcinogens by the Environmental Protection Agency (individual PAH removals up to 80%). The effects of surfactant-enhanced treatment on soil toxicity and genotoxicity were also evaluated. Treatment without surfactant significantly reduced the genotoxicity of the soil while treatment with surfactant had varying effects. Greater PAH removal, however, did not always coincide with a reduction in soil toxicity and genotoxicity. With further optimization of the treatment system, surfactant-enhanced treatment may increase the applicability of bioremediation as means of meeting soil remediation goals.

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TECHNICAL ABSTRACT

Urinary phthalate metabolites in relation to maternal serum thyroid and sex hormone levels during pregnancy: a longitudinal analysis

There is increasing evidence that exposure to phthalates during pregnancy may elevate the risk of adverse reproductive outcomes such as preterm birth. Maternal endocrine disruption across pregnancy may be one pathway mediating some of these relationships. We investigated whether urinary phthalate metabolites were associated with maternal serum thyroid (thyroid-stimulating hormone [TSH], free thyroxine [FT4], and free triiodothyronine [FT3]) and sex (sex hormone-binding globulin [SHBG], progesterone, and estradiol) hormone levels at multiple time points during pregnancy. Preliminary data (n=106) were obtained from an ongoing prospective birth cohort in Northern Puerto Rico. We collected urine and serum samples at 18±2 and 26±2 weeks of gestation. In the longitudinal analyses using adjusted linear mixed models, we observed significant inverse associations between mono-3-carboxypropyl phthalate (MCPP) and FT3 and between mono-ethyl phthalate (MEP) and progesterone. In cross-sectional analyses by study visit, we detected stronger and statistically significant inverse associations at the third visit between FT3 and MCPP as well as mono-carboxyisooctyl phthalate (MCOP); also at the third visit, significant inverse associations were observed between FT4 and metabolites of di-(2-ethylhexyl) phthalate (DEHP). The inverse association between MEP and progesterone was consistent across study visits. In this group of pregnant women, urinary phthalate metabolites may be associated with altered maternal serum thyroid and sex hormone levels, and the magnitude of these effects may depend on the timing of exposure during gestation.

LAY ABSTRACT

Phthalates in relation to maternal serum thyroid and sex hormone levels during pregnancy

Phthalates are a group of manmade chemicals that are commonly used in industrial applications as well as various household and consumer products. Due to their widespread use, human exposure to phthalates is common. We know that exposure to phthalates during pregnancy may elevate the risk of adverse reproductive outcomes such as preterm birth. We have limited information about how phthalates may influence the risk of these unfavorable outcomes. It is possible that phthalates induce changes in maternal hormone levels during pregnancy, which in turn influence subsequent reproductive health endpoints. To determine whether phthalates are related to changes in maternal serum thyroid and sex hormone levels in pregnancy, we used data obtained from the first 106 pregnant women participating in an ongoing birth cohort in Northern Puerto Rico. We collected urine and serum samples from these women at two times points during pregnancy. We found that across pregnancy, increasing levels of certain urinary phthalate metabolites (i.e., the breakdown products of phthalates) were associated with decreasing levels of serum free triiodothyronine [FT3] and progesterone. When we analyzed the data at the first and second time points separately, we observed at the second time point a stronger relationship between increasing levels of certain urinary phthalate metabolites and decreasing levels of serum FT3 and free thyroxine [FT4]. We concluded that in this group of pregnant women, urinary phthalate metabolites may influence maternal serum thyroid and sex hormone levels, and that the extent of these changes may depend on the timing of exposure during pregnancy.

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TECHNICAL ABSTRACT

Identification of OPT4 as a Plant Glutathione Transporter

Glutathione (GSH) is a tri-peptide essential for key processes in plants, including control of the cell redox status, heavy metal transport and resistance, detoxification of xenobiotics, and long-distance transport of organic sulfur. In flowering plants, glutathione is synthesized in the chloroplasts of leaves and then transported to roots and other organs. However, the genes encoding plasma membrane glutathione transporters remain largely unknown. In this study, a screening method to identify plant plasma membrane glutathione transporters was pursued. A screen of the entire family of oligopeptide transporters in Arabidopsis thaliana identified AtOPT4 as a glutathione transporter. When expressed in a heterologous yeast system, AtOPT4 showed plasma membrane glutathione uptake at levels sufficient to complement a yeast sulfur auxotrophic mutant. Radiolabeled GSH transport analyses and confocal microscopy of an OPT4-GFP mutant place OPT4 as a glutathione transporter expressed at the plasma membrane. Root elongation assays of AtOPT4 T-DNA insertion mutants on cadmium and arsenic supplemented media will also be presented.

LAY ABSTRACT

Identification of OPT4 as a Plant Glutathione Transporter

Glutathione (GSH) is a small peptide essential for several processes in plants, including maintaining chemical balance, heavy metal transport and resistance, detoxification of foreign compounds, and long-distance transport of sulfur. In flowering plants, glutathione is synthesized in the leaves and then transported to roots and other organs. However, the genes encoding transporters of GSH remain largely unknown. A screen of the entire family of oligopeptide transporters in the plant, Arabidopsis thaliana, identified AtOPT4 as a glutathione uptake transporter. Further biochemical and kinetic characterization places OPT4 as a glutathione transporter expressed at the surface membrane of cells.

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Environmental Sciences and Engineering

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TECHNICAL ABSTRACT

Environmentally Persistent Free Radical (EPFR) Yields in Dosed PM2.5 and Model PM Samples

Environmentally persistent free radicals (EPFRs) were previously demonstrated to be present in PM2.5 and are known to induce health effects. The general origin, formation mechanism, behavior of EPFRs, and character of their association with particles are discussed in literature. However, the exact chemistry of these intrinsic radicals in PM2.5 as a function of environmental variables—namely organic pollutants, humidity changes, and solar irradiation—is not clear. Our research is focused on three specific aims: (1) understanding radicals behavior in PM2.5 by performing precursor dosage experiments on PM2.5, soot model particles, and surrogate metal particles; (2) understanding how environmental parameters affect sustainability and lifetime of EPFRs by performing decay studies on aforementioned particles under controlled environments; (3) investigating a possible correlation between particle elemental composition and EPFR yields under controlled environment.

LAY ABSTRACT

Environmentally Persistent Free Radicals (EPFRs) in Dosed PM2.5 and Model PM Samples

The human body has a variety of mechanisms for dealing with certain types and concentrations of reactive-oxygen species (ROS) such as superoxide, hydroxyl radical, hydrogen peroxide, etc. Studies demonstrate increased abundance of ROS when PM2.5 (particulate matter, diameter ≤ 2.5 μm) deposits into the lungs. Furthermore, it has been shown that there exist a variety of significant health impacts that are linked to inhalation of PM2.5, including mortality, morbidity, cardiovascular disease, and lung cancer. There is suspicion that radical species are the causative agents. Environmentally persistent free radicals (EPFRs) were previously demonstrated to be present in PM2.5. The hypothesis proposed in this research focuses on achieving an understanding of radicals behavior in combustion, naturally born PM2.5 as well as in model, synthesized particulates under ambient atmospheric conditions (at different humidity's and solar fluxes).

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TECHNICAL ABSTRACT

Aryl Hydrocarbon Receptor (AHR) Polymorphism G1661A: Effects on TCDD-Mediated Biological Activity in the Human B Cell

2,3,7,8-tetraclorodibenzo-p-dioxin (TCDD) is persistent environmental pollutant. TCDD suppresses antibody responses via activation of the AHR, a ligand activated transcription factor. TCDD is known to directly target B cells to suppress antibody responses. We have shown that human B cells exhibit similar sensitivity to TCDD-mediated suppression of the IgM response as "responsive" mouse strains, with the exception that approximately 1 in 6 are refractory to suppression of the IgM response by TCDD. AHR sequencing of "responders" and "non-responders" revealed that a number of non-responders express a G1661A AHR SNP. We hypothesized that loss of sensitivity to TCDD-induced suppression of the IgM response is due to a G1661A polymorphism within the AHR. To test this hypothesis, the AHR null SKW 6.4 human B cell line was transduced to establish SKW-AHR+ and SKW-ΔAHR cell lines that stably express the wild type (WT) or G1661A polymorphic form of the AHR. TCDD-induced Cyp1B1 mRNA expression was lower in SKW-ΔAHR than in AHR+ cells, suggesting decreased transactivation potency of the G1661A AHR. To establish the effects of G1661A polymorphism on TCDD-induced IgM response, LPS-activated SKW cells were treated with increasing concentrations of TCDD and assayed for secreted IgM. Quantification of secreted IgM in response to LPS activation demonstrated comparable suppression by TCDD in both cell lines. Moreover, the slope of the concentration-response curves for TCDD-mediated IgM suppression for SKW-AHR+ and SKW-ΔAHR cells were similar. We conclude that the G1661A human AHR SNP alone did not attenuate TCDD-induced IgM suppression but altered sensitivity to induction of CYP1B1.

LAY ABSTRACT

Effects Of DNA Sequence Variation In Aryl Hydrocarbon Receptor (AHR) On The Sensitivity Of Human Immune System to Suppression by Dioxin

Dioxin is an environmental pollutant known to adversely affect human and animal health. Dioxins directly suppress immune response as evidenced by a decrease in antibody production by white blood cells, termed, B-lymphocytes. Effects of dioxin are mediated by the aryl hydrocarbon receptor (AHR). Interestingly, people differ in their sensitivity to the dioxin-produced suppression of IgM secretion. We hypothesized that small differences (one nucleotide) in DNA code for the AHR gene contribute to differential susceptibility to dioxin immune system toxicity in the human population. We modified a human B cell line that does not express the AHR to express a "wild type" or a polymorphic form of the AHR. We confirmed that AHR is expressed in new cell lines and measured the ability of different forms of the AHR to induce the expression of genes known to be influenced by dioxins and as well as the ability of dioxin to suppress antibody production in these cell lines. We found that wild type AHR is better in inducing gene expression as compared to polymorphic form of the AHR. However, the ability of dioxin to suppress antibody production in both cell lines is similar. We conclude that one change in nucleotide sequence of the AHR is not sufficient to account for the variability to dioxin sensitivity in humans. Additional studies in cells expressing different polymorphic forms of the AHR need to be investigated in order to understand the role of DNA sequence variations in individual sensitivity to dioxin toxicity in the human population.

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TECHNICAL ABSTRACT

Metabolomics of asbestos exposure

Industrial use of asbestos has resulted in a wide range of exposures in human populations. Now known to cause cancer of the lung, the health and economic impacts of asbestos exposure are well documented and being felt by many today. The exceptionally long latency periods of most asbestos related diseases has hampered preventative and precautionary steps thus far. Therefore there remains a large unmet need for the evaluation and quantification of asbestos exposure on an individual basis. Accurate evaluation of exposure levels would aid in identifying at risk individuals as well as determining the effectiveness of remediation strategies. Current biomarkers of asbestos exposure are limited to a small number of proteins. In addition to these proteins, identification and implementation of small molecules biomarkers are likely to contribute to the evaluation of asbestos exposure across human populations. Described here is a study conducted to identify small-molecule biomarkers of asbestos exposure through untargeted metabolomics analysis. There were three groups: healthy controls (C) (n=20), asbestos exposed (A) (n=20), and mesothelioma (M) (n=20). After serum processing by a modified Folch extraction, the organic phase was concentrated and analyzed by high-resolution nanospray LC-HRMS (liquid chromatography-high resolution mass spectrometry) in both positive and negative mode. The software platform SIEVE 2.0 (Thermo Scientific) was used to analyze all data.

LAY ABSTRACT

Metabolomics of asbestos exposure

Industrial use of asbestos has resulted in a wide range of exposures in human populations. Now known to cause cancer of the lung, the health and economic impacts of asbestos exposure are well documented and being felt by many today. The exceptionally long latency periods of most asbestos related diseases has hampered preventative and precautionary steps thus far. Therefore there remains a large unmet need for the evaluation and quantification of asbestos exposure on an individual basis. Accurate evaluation of exposure levels would aid in identifying at risk individuals as well as determining the effectiveness of remediation strategies. Current biomarkers of asbestos exposure are limited to a small number of proteins. In addition to these proteins, identification and implementation of small molecules biomarkers are likely to contribute to the evaluation of asbestos exposure across human populations. Described here is a study conducted to identify small-molecule biomarkers of asbestos exposure through untargeted metabolomics analysis. There were three groups: healthy controls (C) (n=20), asbestos exposed (A) (n=20), and mesothelioma (M) (n=20). After serum processing by a modified Folch extraction, the organic phase was concentrated and analyzed by high-resolution nanospray LC-HRMS (liquid chromatography-high resolution mass spectrometry) in both positive and negative mode. The software platform SIEVE 2.0 (Thermo Scientific) was used to analyze all data.

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TECHNICAL ABSTRACT

Identification and Toxicity of Polycyclic Aromatic Hydrocarbon (PAH) Transformation Products in Bioremediated Soils

The bioremediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs) is a concern due to the potential formation of more toxic oxygenated byproducts. PAHs are persistent in the environment, resistant to biodegradation, and some are known to have carcinogenic and mutagenic properties. Their oxygenated derivatives are more likely to be mobile in the environment, and could pose greater health risks. This study utilizes an effects-directed approach, combining toxicity analysis using the novel DT40 chicken bioassay, and gas chromatography mass spectrometry (GC/MS) for chemical analysis. The purpose of the study is to isolate and identify compounds in soil, which could potentially be the cause for increased toxicity in soil post bioremediation.
We developed an extraction method using pressurized liquid extraction (PLE), and fractionated the contaminants in the soil using silica solid phase extraction (SPE), by varying solvent elution polarity. The DT40 bioassay was then used to assess the toxicity of the different fractions. We observed an increase in the overall toxicity of the soil, and an increase in genotoxicity and mutagenicity in certain fractions post bioremediation.

LAY ABSTRACT

Investigating the toxicity and fate of PAHs in soil post bioremediation

Polycyclic aromatic hydrocarbons (PAHs) are a type of contaminant released through the incomplete combustion of organic matter. Bioremediation is used to treat soils contaminated with PAHs, but it's possible that these compounds could become more toxic through bioremediation. The increase in toxicity is possibly as a result of these compounds forming compounds that are more toxic and available after bioremediation.
We investigated soil toxicity and the potential formation of toxic byproducts during the bioremediation of contaminated soil. We developed a method where we studied the soil toxicity and PAH composition before and after the bioremediation. To help with identifying these toxic compounds, the soil was separated into different portions based on polarity before analysis. We confirmed an increase in toxicity in the overall soil, and in certain portions of the soil after bioremediation.

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TECHNICAL ABSTRACT

Evaluation of different nanoparticle support systems for detoxification of chlorinated organic compounds

Water pollution by chlorinated organic compounds is a serious concern due to their toxic effect. One of these compounds, Trichloroethylene (TCE), is stored in body fat and can cause damages in liver and kidney. TCE has low biodegradability and small quantities are found in ground water.
Various studies have showed the effect of nano-structured materials in the decontamination of water by TCE. These materials prevent the loss of nanoparticles and have high surface areas that increase their reactivity. In order to improve dechlorination in water, it is necessary to explore different platforms such as functionalized hydrogels or hollow fiber/spongy microfiltration membranes. The present work aims this, using hydrogels of poly (acrylic acid) (PAA) and membranes of polyvinylidene fluoride (PVDF)-PAA to immobilize Fe/Pd nanoparticles to treat aqueous solutions of TCE.
Quantitative TCE/chloride generation by Fe/Pd nanoparticles in hydrogels and membranes were performed in batch process. Iron nanoparticles (30-120 nm) followed pseudo-first order reaction kinetics with higher surface-area constant using hydrogels (2.46 L/ (m2×h)) than using membranes (0.02 to 0.13 L/ (m2×h)). Similar results between hydrogel and solution phase dechlorinations were found. 80% of TCE degradation was achieved in membranes. Produced chloride is close to the stoichiometric value, indicating negligible production of intermediates. In the PAA-hydrogel, 450 mgFe/gPAA were produced as Fe/Pd nanoparticles. Comparative analysis of iron loading between platforms from previous and current works were also made.
This research is financed and supported by the NIEHS-SRP grant P42ES007380. Hollow fiber Membranes were synthesized at the Singapore Membrane Technology Center, NTU, Singapore.

LAY ABSTRACT

Evaluation of different nanoparticle support systems for detoxification of chlorinated organic compounds

Water pollution by chlorinated organic compounds is a serious concern due to their toxic effect in humans. One of these compounds, Trichloroethylene (TCE), is stored in body fat and can cause damages in liver and kidney. Small quantities of this contaminant are found in ground water. For these and other reasons, its treatment is necessary.
Various studies have showed the effect of nano-structured materials in the decontamination of water by TCE. These materials prevent the loss of nanoparticles and have high surface areas that increase their reactivity. In order to improve TCE treatment in water, it is necessary to explore different platforms such as functionalized hydrogels or hollow fiber and spongy microfiltration membranes. The present work aims this, using hydrogels of poly (acrylic acid) (PAA) and membranes of polyvinylidene fluoride (PVDF)-PAA to immobilize iron-palladium nanoparticles and treat aqueous solutions of TCE.
The TCE treatment with iron nanoparticles of 30-120 nm sizes was compared between these platforms, finding similar results between hydrogel and solution phase dechlorination, and an 80% of TCE degradation in a membrane domain. Dissolved chloride produced due to detoxification was also measured, finding its value very close to the chlorine within the TCE molecule in the initial solution (3:1). In hydrogel alone, 450 mgFe/gPAA were produced as iron-palladium nanoparticles. Comparative analysis of iron loading between platforms from previous and current works were also made.
This research is financed and supported by the NIEHS-SRP grant P42ES007380. Hollow fiber Membranes were synthesized at the Singapore Membrane Technology Center, NTU, Singapore.

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TECHNICAL ABSTRACT

Impacts of particle-based dispersants on benzene bioavailability and on toxicity using Artemia franciscana as a model organism

Currently, a common emergency response method for off-shore petroleum spills is the use of synthetic chemical dispersants. It is known that the chemical dispersants are moderately toxic to marine organisms, however they are still implemented because the toxicity of the petroleum is generally regarded as the more significant threat. Fine carbon particles with engineered surface chemistry have been shown to stabilize oil-in-water emulsions, but the environmental impacts of large-scale particle introduction to the marine environment are unknown. Both particle and chemical based dispersants serve to limit petroleum accumulation at the ocean surface and accelerate biodegradation processes by increasing the oil-water interfacial area. The potential advantage of our carbon black particle –based Pickering emulsion is the ability to not only stabilize droplets but also to adsorb the more water-soluble and toxic fractions of petroleum. Benzene is used as a model aromatic compound to determine the efficacy of the carbon black particles to adsorb and retain (therefore reducing the bioavailability) of these fractions as a function of tunable hydrophilicity. Artemia franciscana (brine shrimp) larvae are used as a model marine microcrustacean to assess toxicity. Endpoints for the larvae include activation of stress responses, oxidative damage, reduced motility and death. This study provides a novel, interdisciplinary approach for rational design of surface-engineered nanoparticle-based dispersants and evaluation of their potential impacts on marine organisms.

LAY ABSTRACT

Engineering safer dispersants for use in off-shore oil spills

Currently, a common emergency response for off-shore petroleum spills is the use of chemical dispersants. These work by breaking the oil slick into small droplets, thereby increasing the oil-water interfacial area and allowing microorganisms better access for biodegradation. It is known that these dispersants are moderately toxic, but are still implemented because the toxicity of the petroleum is regarded as a more significant threat. A potential alternative is to use nanoparticles, which are able to assemble at water-oil interfaces and stabilize fine oil droplets. Carbon-based particles are also capable of lowering the concentration of soluble, toxic fractions of petroleum in the water through a process known as adsorption. This project studies the efficacy of the different engineered carbon black particles to adsorb and retain (therefore reducing the bioavailability) benzene, a model water-soluble petroleum compound. Brine shrimp (Artemia franciscana) are used as a model marine microcrustacean to assess toxicity. This is an interdisciplinary study aimed at designing and evaluating new class of high-efficiency, low-toxicity, particle-based alternative dispersants.

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TECHNICAL ABSTRACT

Sulfated Metabolites of Polychlorinated Biphenyls are Toxic to Rat N27 Neuronal Cells

While the legacy of polychlorinated biphenyl (PCB) contamination and toxicity is well known, lower chlorinated-PCBs are of particular current interest due to their semi-volatile properties and potential for metabolic conversion to toxicologically active compounds. Epidemiological as well as in vitro/vivo studies show that PCB exposure is linked to neurodevelopmental and neurodegenerative disorders related to the dopaminergic system. Recent studies in rats have shown that the formation of sulfate esters is a significant in vivo metabolic pathway for PCB 3. Moreover, PCB sulfates bind with congener-specific affinities to the two major drug-binding sites in human serum albumin (HSA), potentially serving in the retention and/or distribution of PCB sulfates. We hypothesize that, similar to parent and OH-PCBs, PCB sulfates are toxic to dopaminergic cells, and that the presence of HSA affects this toxicity. The N27 rat dopaminergic neural cell line was used to assess the toxicity of the 4-PCB sulfates derived from PCBs 3, 8, 11, 12, and 52 in comparison to their corresponding OH-PCBs. Our preliminary results show that these PCB sulfates were cytotoxic to N27 cells (MTT assay). Although 4-PCB 52 sulfate showed increased toxicity when compared to 4-OH-PCB 52, other PCB-sulfates were less cytotoxic than the corresponding OH-PCBs. The presence of HSA in the cell culture medium modulated the toxic effects of these compounds. These results indicate that PCB sulfates may play a role in PCB-induced neurotoxicity and that binding to HSA may influence this effect. [Supported by NIH P42 ES013661 and by R25GM058939]

LAY ABSTRACT

Sulfated Metabolites of Polychlorinated Biphenyls are Toxic to Rat N27 Neuronal Cells

The harm that man-made environmental toxins exert on biological and environmental systems is a great concern for our planet, and one class of compounds that has been of considerable interest is the polychlorinated biphenyls (PCBs). Exposure to these agents has been associated with various disorders and diseases, some of which involve development and function of the brain. Although overall PCB levels have decreased worldwide in the last few decades, those PCBs with lower numbers of chlorine atoms have garnered increasing interest due to their prevalence in indoor and outdoor air. These PCBs undergo metabolic changes in the body, and one metabolic pathway results in the formation of hydroxylated and sulfated derivatives. A sulfate group may make the PCB more readily excreted, but it may also enable its transport to tissues through binding to proteins such as serum albumin. Since we are interested in the potential toxicities of these metabolites to cells in the brain, we have initially examined their effects on the viability of rat-derived neuronal cells. We found that PCB sulfates were toxic to these neuronal cells. The phenolic metabolites were generally more toxic than the sulfates, however this was not true in one case tested. Addition of human serum albumin to the cells altered the effects of these compounds. Thus, this is a first step in discerning the contribution that sulfation may have in PCB associated neurotoxicity, as well as the role that binding to serum proteins may play in it.

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TECHNICAL ABSTRACT

Integrating Differential Gene Expression with Hepatic, Serum, and Urinary Metabolomes Identifies TCDD-elicited Interactions Between Disrupted Metabolic Pathways

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) elicits hepatic lipid accumulation that can progress to steatohepatitis with fibrosis in mice. To further investigate the hepatic effects of TCDD, female C57BL/6 mice were orally gavaged every 4 days for 28 days with sesame oil vehicle, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, or 30 ug/kg TCDD. RNA-Sequencing identified 1,294 unique differentially expressed hepatic genes (|fold-change| ≥ 2.0, P1(t) ≥ 0.8) associated with oxidative stress, energy metabolism, immune response, and fibrosis. Targeted LC-MS/MS analysis detected 126, 72 and 130 metabolites in hepatic extract, serum and day 26 urinary metabolomes, respectively (vehicle, 3, 10 and 30 ug/kg only). Cytoscape was used to map differential expression and metabolite data onto 15 disrupted KEGG pathways that converged to carbohydrates, NADPH and acyl-CoAs. For example, decreased acyl-CoA levels and the concomitant down-regulation of acetyl-CoA acyl transferase (thiolase) and acyl-CoA synthetase (acetyl-CoA carboxylase) gene expression are indicative of β-oxidation inhibition and consistent with hepatic lipid accumulation. Furthermore, limiting acetyl-CoA production from β-oxidation compromises the TCA cycle resulting in an increase in oxaloacetate levels and impaired cholesterol biosynthesis. Collectively, integration of transcriptomic and metabolomic data suggests TCDD elicits systemic metabolic dysregulation associated with the dose-dependent progression of steatosis to steatohepatitis with fibrosis.
Funded by SRP P42ES04911.

LAY ABSTRACT

Integration of Gene Expression and Metabolite Levels Identifies Metabolic Pathways Disrupted by TCDD

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) causes fat accumulation in the liver which can progress to inflammation and fibrosis, effects widely known as non-alcoholic fatty liver disease (NAFLD). We investigated this metabolic disruption by simultaneously measuring expression changes in thousands of genes or metabolites after TCDD treatment in mice. Gene and metabolite changes caused by TCDD were mapped to known 15 pathways involved in glucose and fat metabolism, two key players in NAFLD development. For example, a number of involved in fat metabolism (β-oxidation pathway)were expressed at lower levels consistent with decreases in fat breakdown products and the accumulation of fat in the liver. β-oxidation is also an important source of acetyl-CoA which can be used by the citric acid cycle (TCA cycle) and cholesterol synthesis pathways, both of which also show evidence of disruption by TCDD exposure. Considering these pathways together implicates acyl-CoAs as a central component to metabolic disruption by TCDD. The integration of results from these large scale studies has not only provided more information regarding the toxicity of TCDD and related compounds, but also identified a target that can be used to develop drugs for the treatment of NAFLD as well as more complex diseases such as liver cancer, cardiovascular disease and diabetes.
Funded by SRP P42ES04911.

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TECHNICAL ABSTRACT

Molecular alterations in sperm are sensitive indicators of testicular dysfunction

Traditional endpoints used to measure male reproductive toxicity in humans, including semen and hormone analysis, are insensitive and unreliable; those used to monitor toxicity in animal studies, while sensitive, are not easily translatable to humans. It is therefore necessary to develop sensitive and reliable molecular biomarkers of testicular injury that can be used to both monitor human reproductive function and compare animal studies with human exposures. We approached this problem by exposing male rats to model testicular toxicants to identify sperm molecular alterations, as these can be compared to highly sensitive histopathological assessments of testicular function. Adult male rats were exposed to cyclophosphamide (CPP) for 12 weeks (1.4, 3.4, or 5.1 mg/kg/day p.o.) or 12 weeks plus a recovery period of 12 weeks (5.1 mg/kg/day p.o.) as a model of germ cell toxicity. Standard reproductive endpoints were examined; in particular, germ cell apoptosis and spermatid head retention were quantified as sensitive markers of damage. mRNA from cauda epididymidal sperm was analyzed for toxicant-induced alterations using a genome-wide microarray, then significant and robust alterations were further examined using qRT-PCR arrays and standard qPCR. We observed that CPP produced dose-dependent testicular injury that resolved after a 12-week recovery period. The levels of injury correlated with specific changes in transcript abundance, indicating a utility for these mRNAs as translatable biomarkers for male reproductive dysfunction. These transcripts will be examined in additional exposure settings, as well as both fertile and subfertile men to continue to validate the relevance of these alterations.

LAY ABSTRACT

Sperm biomarkers of male reproductive function

There are many chemicals and pharmaceutical drugs that cause damage to the male reproductive system when given at high levels or for long periods of time. In such a situation, whether by accidental environmental exposure or as prescribed by a doctor, it is important to monitor reproductive function of affected men. Unfortunately, the ways in which doctors can do this are limited and unreliable. It is also difficult to compare human exposures to tightly controlled scientific experiments because there are major differences in reproductive monitoring between men and laboratory animals. Researchers and doctors would benefit greatly from a reliable biological measurement (a “biomarker”) of male reproductive function and fertility that applies to both men and laboratory animals. We proposed sperm as the source of these biomarkers; to test this, we exposed animals to a common chemotherapy drug and collected sperm after three or six months. We found that levels of certain mRNAs, which are sensitive readouts of DNA, were different in sperm from exposed versus unexposed animals. We think that these mRNAs are promising biomarkers, and with further research using both additional chemicals and human sperm, we can determine whether they are useful for a wider range of toxic exposures in both animals and humans.

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TECHNICAL ABSTRACT

LAY ABSTRACT

Characterization of asbestos exposure and associated mortality among residents of a small town.

Introduction: Chronic occupational asbestos exposure is linked to increased rates of both benign and malignant lung disease. There are also studies linking paraoccupational exposure to mesothelioma. However, few studies have quantified the extent of contributions from non-occupational, environmental exposure. Our study aims to comprehensively characterize asbestos exposure and its related mortality.
Methods: This is a retrospective cohort study of individuals residing near the asbestos industry in Ambler, Pennsylvania during the 1930s-1940s. Using Census data, individuals will be classified by age, sex, occupation, income, source of residential drinking water, and residential location relative to the asbestos plant. Historic climate data will be accessed to determine prevailing wind trends in Ambler in the 1930s-1940s to estimate disproportionate exposure to plant exhaust downwind. Using data from the National Death Index and life tables, the mortality of Ambler residents will be compared to their expected mortality (relative to matched controls).
Results: This presentation will report mapped geographic information describing the built and social environment in the Ambler, PA study area, demographic characteristics of sample and variability in paraoccupational and environmental exposure to asbestos. Future study phases will also be described, including steps involved in identifying subjects who died prematurely and in conducting a statistical analysis to identify geographic factors associated with premature death.
Conclusions: The results of this study will provide valuable information about the range of mechanisms through which asbestos exposure occurs, the spatial distribution of health effects that result from these mechanisms, and a quantitative measure of mortality associated with asbestos exposure.

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TECHNICAL ABSTRACT

Improving power to detect fold-changes in blood miRNA expression by accounting for sources of variability in the experimental design

Blood microRNAs (miRs) biomarkers are a new promising area of toxicology research, but variability in miR measurements may limit detection of true-positive findings. Here, we measured sources of miR variability and determine whether repeated measures can improve power to detect fold-change differences between comparison groups. Blood from healthy volunteers (N=12) was collected at three time points. The miRs were extracted by a method predetermined to give the highest miR-yield. Nine different miRs were quantified using different qPCR assays and analyzed using mixed models to identify sources of variability. Separately, a publicly-available blood miR microarray dataset with repeated measures was used for bootstrap simulations to investigate effects of repeated-measures on power to detect fold-changes in miRNome expression for a theoretical case-control study. Technical variability in qPCR replicates was identified as a significant source of variability ($p<0.05$) for all nine miRs tested. Variability was larger in the TaqMan qPCR assays (SD = 0.15-0.61) versus the qScript qPCR assays (SD = 0.08-0.14). Inter- and intra- individual and extraction variability also contributed significantly for two miRs. The bootstrap-simulation demonstrated that repeated measures (20-50% of N) increased detection of a 2-fold change for ~10-45% more miRs. Statistical power to detect small-fold changes in blood miRs can be improved by accounting for sources of variability using repeated measures and choosing appropriate methods for miR quantification. These identified sources of variability should be included in toxicological and molecular epidemiological studies measuring specific miR levels in biological samples. (Supported by NIEHS P42ES004705 to MTS)

LAY ABSTRACT

Improving detection of small differences in miRNA levels by including repeated measures

Blood microRNAs (miRs), short pieces of RNA that regulate protein synthesis, act as biomarkers in humans. Researchers have already identified specific miRs that are directly associated with certain diseases and environmental exposures, but their results are difficult to confirm in separate populations. This may be because previous experiments did not account for technical variability that occurs during processing of the samples, such as during extraction and quantification of miRs. Here, we measured both sources of 'unwanted' technical variability and real biological variability within and between people. Over the course of 8 months, we took blood samples at three time points from each of 12 healthy volunteers to see if miR levels within and between people vary over time. We repeated the extraction and measuring procedures to examine how blood miR levels vary due to handling and precision of the assay. We found that the variability in miR quantification contributed to a majority of the total variability for several miRs tested. The healthy volunteer results and publically available data on miR variability were used to show how including replicates could help improve detection of these small, yet still important, fluctuations for miR levels in a hypothetical human study. This demonstrated the importance of using replicate measures in miR experiments for human population studies in the future.

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TECHNICAL ABSTRACT

LAY ABSTRACT

Evidence of Differences in Toxic Responses to a Common Contaminant (PCB-126) in Atlantic Killifish in the Lower Mystic River Watershed, Chelsea, MA

Fish consumption advisories exist in many contaminated water bodies. Efforts to reexamine the fish consumption advisory in the Lower Mystic River (LMR) Watershed, an urban watershed near Boston, highlight the need to understand links between ecological and human health impacts of contaminants. Industrial pollutants, such as some polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) can cause harm to developing embryos of Atlantic killifish (Fundulus heteroclitus), a small fish living along the east coast of the United States. However, killifish living in highly contaminated locations can become resistant to the toxic effects of these pollutants, requiring a higher amount of contaminant to cause harm in resistant fish compared to non-resistant fish. Resistance may also lead to higher amounts of pollutants in killifish and their predators. Killifish have transparent embryos that are ideal for studying the effects of pollutants on development, since a microscope can be used to observe important developmental processes, such as heart formation, which can be impaired by exposure to PCBs and PAHs. In this study we examined whether killifish living in the LMR watershed, were resistant to PCBs and PAHs. To answer this question we exposed embryos from the LMR and an uncontaminated site, Scorton Creek (SC) to a PCB and PAH-like compound and looked for differences in genetic material and heart formation. IER killifish show signs of resistance to PCBs and PAHs compared to those from SC. This finding allows us to conduct further research about how such resistance could impact human health through fish consumption. [Supported in part by NIH grant P42ES007381.]

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TECHNICAL ABSTRACT

LAY ABSTRACT

Exposure To Common Flame Retardants Can Lead To Early-Life Hyperactivity In Animal Models

The use of organophosphate (OP) flame retardants is growing rapidly and studies have found human exposure to be widespread and significant. Although a chemically similar class of OP pesticides have been shown to disrupt the development of the brain in both humans and animal models, the potential for OP flame retardants to be similarly harmful is relatively unexplored. This study was conducted to compare the effects of developmental exposure of two common OP flame retardants (TPP and TDCPP) to the OP pesticide chlorpyrifos (CPF) in an animal model of activity levels. Zebrafish were exposed to a range of concentrations of CPF, TPP, or TDCPP from 0-5 days post fertilization via immersion. Following exposure, zebrafish larvae were placed in clean aquarium water for 24 h. We then tested the young fish in a swimming test consisting of two cycles of illumination and darkness. All 3 OP compounds were found to cause hyperactivity in the swim test. However, the timing of the hyperactivity over the course of the test differed between the OP pesticide and OP flame retardants. While CPF only caused hyperactivity during the first half of the test, the OP flame retardants had no effect on the first half yet caused hyperactivity during the latter half. Additionally, while TPP was toxic at the same concentration as CPF, TDCPP began causing effects at one-tenth of the dose used for CPF. These findings suggest that OP flame retardants do have significant effects on behavior and may be significant environmental contaminants.

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TECHNICAL ABSTRACT

Genomic models of short-term exposure accurately predict long-term carcinogenicity and identify putative mechanisms of action

Despite an overall decrease in incidence of and mortality from cancer, about 40% of Americans will be diagnosed with the disease in their lifetime, and around 20% will die of it. Current approaches to test carcinogenic chemicals adopt the 2-year rodent bioassay, which is costly and time-consuming. As a result, fewer than 2% of the chemicals on the market have actually been tested. However, evidence accumulated to date suggests that gene expression profiles from model organisms exposed to compounds reflect underlying mechanisms of action, and that these toxicogenomic models could be used in the prediction of carcinogenicity.
We used a rat-based microarray dataset from the NTP DrugMatrix Database to test the ability of toxicogenomics to model carcinogenicity. We analyzed 1,221 gene-expression profiles obtained from rats treated with 127 well-characterized compounds and built a classifier that predicts a chemical's carcinogenic potential, and validated it on an independent dataset consisting of 2,065 profiles from 72 compounds. We confirmed and expanded upon previous studies implicating DNA damage, the peroxisome proliferator-activated receptor, the AhR receptor, and regenerative pathology in the response to carcinogen exposure.
Our results validate the toxicogenomic approach to predict carcinogenicity, show that the prediction of carcinogenicity is tissue-dependent, and provide evidence that, with a larger set of compounds, we would be able to substantially improve the prediction performance. For that we are currently translating our findings to human in-vitro systems, where we just analyzed 160 compounds in multiple cell types and at multiple doses using gene expression profiling paired with high-throughput cytological profiling.

LAY ABSTRACT

Genomics is able to identify cancer causing chemicals and underlying biological mechanisms

Despite an overall decrease in cancer, about two in five of Americans will be diagnosed with the disease in their lifetime, and every fifth will die of it. Currently, 2-year long rat tests are used to determine whether or not a chemical causes cancer, which is expensive and takes a long time. As a result, less than one in fifty of the chemicals on the market have actually been tested. However, current research suggests that we can capture the biological changes that happen when rats or cell lines are exposed to a carcinogen by looking at gene expression and use them to test whether a compound potentially causes cancer.
In this study, we used a large-scale rat dataset from the National Toxicology program, the DrugMatrix Database, which includes gene-expression data from 1,221 rats treated with 127 well-known compounds. We built a model that is able to predict whether a chemical is a carcinogen, and validated it on an independent dataset that contains profiles from another 2,065 rats that were treated with 72 different chemicals. We confirmed and expanded upon previous studies, which includes the importance of damage to DNA and the activation of the aryl hydrocarbon receptor, a key player in environmentally caused cancer.
Our results show that gene expression data can be used to find carcinogens, show that different chemicals cause cancers in different tissues and provide evidence that, with a larger set of chemicals we would do an even better job at finding carcinogens accurately.

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TECHNICAL ABSTRACT

LAY ABSTRACT

Influence of Air Pollution on Infant Bronchiolitis Hospitalization

Particulate matter less than 2.5μm in diameter ($PM_{2.5}$) can alter immune function making individuals, especially infants, more susceptible to illness. Outdoor air $PM_{2.5}$ is a mixture of particles from vehicle exhaust emissions, industrial activities, and coal and wood burning, and levels are typically highest in the winter. The goal of this work is to determine if short-term exposure to $PM_{2.5}$ is associated with infant bronchiolitis, the leading cause of hospitalizations in the first year of life, among all infants born in Massachusetts between 2001and 2009. Average daily $PM_{2.5}$ concentrations for all of Massachusetts were modeled using satellite remote sensing data. We analyzed 11,805 hospitalization records of infant bronchiolitis using a case-cross over study design where cases serve as their own controls. Using this study design, only variables that change during the short-term, such as the temperature and humidity, need to be considered in the analyses along with exposure. Results indicate that for an additional 10 μg increase of $PM_{2.5}$, infants were 6 to 28% more likely to be hospitalized for bronchiolitis, depending on gestational age and seasonality. Infants born prematurely between 32 and 37 weeks of gestation were more likely to be hospitalized for bronchiolitis than infants born after 37 weeks gestation. The influence of $PM_{2.5}$ on bronchiolitis risk was stronger during the winter months. Understanding the role of $PM_{2.5}$ in terms of increased susceptibility to illness is important for adopting preventive measures. This work can provide evidence to support regulations when determining safe levels of $PM_{2.5}$ for infants.

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TECHNICAL ABSTRACT

Predicting joint effects of PPARγ ligands using Generalized Concentration Addition

Dose (or concentration) addition is a null model used to test hypotheses about the effects of mixture exposures on nuclear-receptor activation. Although it is applicable to modeling effects of mixtures containing ligands with shared curve maxima (e.g. two full agonists), it cannot predict joint effects of full and partial (sub-maximal efficacy) agonist combinations. We previously developed the Generalized Concentration Addition (GCA) model to address this scenario with aryl hydrocarbon receptor activation. Here, we implemented a transactivation assay using a mouse peroxisome proliferator-activated response element (PPRE)-dependent luciferase reporter to determine joint effects of binary mixtures of full and partial peroxisome proliferator-activated receptor gamma (PPARγ) ligands (rosiglitazone, non-thiazolidinedione partial agonist (nTZDpa), mono-2-ethylhexyl phthalate (MEHP)). We then tested the GCA prediction, based on the individual chemical dose response curves, versus the empirical receptor activation by the mixtures. We demonstrate that GCA adequately models the response surface of a binary mixture activating PPARγ and highlight limitations of the Toxic Equivalency Factor and Effect Summation approaches to full and partial agonist mixtures. Further, we show that the efficacy of a dual RXRα/PPARγ ligand (the environmental contaminant tributyltin (TBT)) for PPRE transactivation is unaltered by increasing concentrations of a PPARγ partial ligand. We extended these results to test the ability of the GCA model to predict lipid accumulation (quantified by Nile Red fluorescence) in mouse mesenchymal stromal cells, a downstream effect of PPARγ activation, in response to these ligand mixtures. These studies support the application of GCA in predicting combinatorial toxicant effects from independent dose-response curves.

LAY ABSTRACT

Investigating chemical mixtures: how do toxic effects add up?

As toxicologists, we are interested in determining how chemical exposures cause harm. We often ask questions like: how is an adverse health effect of a chemical related to a person's exposure to that chemical? Does the severity of the chemical's effect increase as the exposure increases? What if there are multiple chemical exposures – can one chemical influence the effect of another? This last question is particularly relevant, as we are exposed to a variety of chemicals through our diet, daily activities, and medications. To explore what we call 'mixture effects' – combined effects of simultaneous chemical exposures – we conduct experiments allowing us to observe the actions of chemicals in cells and graph how the chemical effect changes as we increase the dose. We can graph these effects with a mixture by adding multiple chemicals to the cells at the same time. However, as we add more chemical combinations, the experiments become more challenging. The ability to predict these graphs without experimentally testing every possible chemical combination would solve this problem and be a powerful tool in addressing gaps in our knowledge of chemical safety; each year thousands of chemicals are used with little information on their toxic effects. Our research presents ideas about how chemical mixtures behave in cells and the experiments performed to support or refute these ideas. Ultimately, this work will be used to build predictive screening models – efficient, low cost assays – that reflect a more accurate picture of the large number of chemicals to which we are exposed.

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TECHNICAL ABSTRACT

A mixture of PCB 118, 126, and 153 differentially promotes endothelial cell dysfunction by upregulating inflammatory markers

In addition to individual environmental pollutants, environmentally relevant pollutant mixtures need to be examined to understand the complexity of human exposure and health risks. Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), have been correlated to multiple inflammatory related diseases, including cardiovascular disease. Due to the real world relevance of mixtures and the limited cellular data available, a combination of a coplanar (126), noncoplanar (153) and mixed congener (118) PCBs were utilized; these persistent PCBs were chosen because they are detectable in human serum at significant levels. Porcine primary endothelial cells were exposed to individual PCBs (118, 126, 153), binary PCB mixtures, or a triad congener mixture. Using our model, which is based on three-way ANOVA statistical analyses, we observed a congener specific upregulation of inflammatory markers. Using real time PCR we examined relative mRNA levels of multiple pro-inflammatory markers, including vascular cellular adhesion molecule-1 (VCAM-1), monocyte chemoattractant protein-1 (MCP-1), and cytochrome P450, 1A1 (Cyp1A1). The development of this experimental and statistical model will allow us to investigate the effects of PCB mixtures as well as other pollutants at environmentally and physiologically relevant concentrations. With a fully developed statistical model we can more accurately examine the possible synergistic or antagonistic effects of mixtures of environmental pollutants. Our data suggest that mixtures of PCB congeners contribute to different patterns of induction of inflammatory markers compared to individual congeners. (NIH/NIEHS P42ES007380)

LAY ABSTRACT

A mixture of environmental pollutants promotes the risk for heart disease

Polychlorinated biphenyls (PCBs) are a group of related pollutants with at least 200 individual compounds. Previous research, which was focused on exposures to single PCBs, demonstrated a link between these compounds and cardiovascular disease risk, but little is known about the effects of these compounds as mixtures. In general, humans are exposed to many environmental pollutants, including mixtures of different types of PCBs. Therefore, PCB toxicity in relation to human exposure should also examine these compounds as mixtures, at environmental and physiologically relevant concentrations. Thus, we treated cells that line blood vessels (endothelial cells) with a mixture of three different PCBs detectable in human blood and examined pro-inflammatory markers, which are used as an indicator for cardiovascular disease risk. We determined that, compared to individual PCBs tested, mixtures of these PCBs had unique effects on markers of vascular disease risk. More importantly, we are using these data in conjunction with statisticians to help develop a model that can more appropriately examine complex effects of mixtures of PCBs relative to disease risks.

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TECHNICAL ABSTRACT

Developmental Exposure to Polycyclic Aromatic Hydrocarbons (PAHs) Affects Behavior and Energetics in Larval and Adult Zebrafish

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants present in urban air, dust, and soil resulting from incomplete combustion of organic materials or fossil fuels. It is widely recognized that PAHs pose risks to human health, especially for the developing fetus and infant. Using the zebrafish model, we evaluated the developmental toxicity of PAHs and oxygenated PAHs (OPAHs). Zebrafish embryos were exposed from 6-120 hours post fertilization (hpf) to B[a]P, DB[a,l]P, Ba[A]Q, BEZO, and 9,10-PHEQ concentrations that caused no observable developmental malformations. Using the Seahorse Extracellular Flux Analyzer, we measured in vivo respiration in 26 hpf embryos exposed to PAHs. Exposure to B[a]P, BEZO, and 9,10-PHEQ caused decreased oxygen consumption rates, indicative of mitochondrial damage. Developmental B[a]P and Ba[A]Q exposure also resulted in hyperactive swimming at 120 hpf. To determine if behavioral and physiological effects persisted, a subset of exposed animals were raised to adulthood in chemical-free water and assessed for learning and fitness deficits. Preliminary results indicated B[a]P-dependent deficiencies in learning an active avoidance paradigm. A swim tunnel respirometer was used to measure total oxygen consumption as a measure of adult cardiovascular fitness. Animals developmentally exposed to B[a]P, DB[a,l]P, Ba[A]Q, BEZO, and 9,10-PHEQ demonstrated a significant increase in oxygen consumption rates over control animals during strenuous exercise. These data demonstrate that developmental PAH exposure results in important, non-cancer endpoints and we can begin to identify the mechanisms underlying these complex responses. This research was supported by the NIH grants P42 ES016465 and P30 ES000210.

LAY ABSTRACT

Environmental Contaminants in Juvenile Zebrafish Affect Behavior and Physical Fitness in Adulthood

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants present in urban air, dust, and soil. PAHs are produced by burning common carbon containing materials like wood, gas, oil, and tobacco. We know these PAHs are a health risk; some cause cancer and birth defects. They have also been linked to preterm mortality, lower intelligence, and cardiovascular problems. Exposure to PAHs during fetal development and infancy is of particular concern because these life-stages are often more vulnerable to chemical toxicities. Using the zebrafish as a model system, we studied the toxicity of environmental PAHs and oxygenated PAHs (OPAHs) during a 120 hour period of development, from egg to larvae. Larvae exposed to some of the PAHs had a hyperactive swimming pattern and difficulties properly using oxygen, affecting the metabolism of the animal. When these same fish reached adulthood we saw that they used more oxygen during exercise than unexposed fish and performed poorly in a learning test compared to unexposed animals. Our data provide important new information about how PAHs and oxygenated PAHs may affect learning and physiology in vertebrates. Follow-up studies will attempt to identify how these chemicals cause these effects. This research was supported by the NIH grants P42 ES016465 and P30 ES000210.

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TECHNICAL ABSTRACT

Regulation of GnRH receptor by androgens in LβT2 immortalized gonadotrope cells and a pituitary-specific androgen receptor knockout mouse

Androgens, which act through androgen receptor (AR), are critical for many aspects of normal male reproduction including sexual differentiation and spermatogenesis. In the LβT2 immortalized pituitary gonadotrope cell line, androgen treatment increases activity of the gonadotropin-releasing hormone receptor (GnRHR) promoter. This induction of GnRHR gene expression requires both the ligand-binding and DNA-binding domains of AR, and is achieved by direct binding of AR to hormone response elements located at -159 bp and -499 bp in the GnRHR promoter. To further investigate the role of pituitary AR in male mice, we utilized a Cre-loxP strategy to selectively delete AR from gonadotropes and thyrotropes (PitARKO). Male PitARKO mice experience delayed puberty, but exhibit normal sperm count, serum hormone levels, and copulatory behavior. To examine whether loss of pituitary AR affects GnRHR mRNA, we performed qRT-PCR. In wild-type mice, GnRHR mRNA is significantly reduced when androgen levels are decreased by castration, but this effect of castration is not observed in PitARKO mice, suggesting a physiological role of AR in regulating GnRHR gene expression. Ongoing experiments will determine whether GnRHR mRNA is regulated by AR in primary pituitary cell culture. Understanding the role of pituitary AR in male reproductive physiology will provide insight into the mechanisms through which environmental androgenic compounds may disrupt normal endocrine function.

LAY ABSTRACT

Androgen action in the pituitary

Androgens, such as testosterone, act through the androgen receptor (AR) and are critical for many aspects of normal male reproductive function including sexual differentiation and sperm development. In males, androgens are produced by the testis in response to pituitary hormones, which in turn are produced in response to secretion of gonadotropin-releasing hormone (GnRH) from a specific population of neurons in the brain. GnRH and its receptor, GnRHR, are essential for fertility. In a pituitary cell line, androgen treatment increases activity of the GnRHR gene. This induction of GnRHR gene expression requires direct binding of AR to DNA at regulatory regions in the GnRHR gene. To investigate the physiological role of pituitary AR, we generated mice in which AR is selectively deleted from the pituitary (PitARKO). Male PitARKO mice experience delayed puberty, but exhibit normal sperm count, hormone levels, and sex behavior. We measured amounts of GnRHR gene product in these mice to determine the effect of AR deletion. In wild-type mice, GnRHR is significantly reduced when androgen levels are decreased by castration, but this effect of castration is not observed in PitARKO mice, suggesting a physiological role of AR in regulating GnRHR gene expression. Understanding the role of pituitary AR in male reproductive physiology will provide insight into the mechanisms through which environmental androgens may disrupt normal endocrine function.

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TECHNICAL ABSTRACT

Trichloroethylene toxicokinetics and toxicodynamics in wild-type, PPARalpha-null and PPARalpha-humanized mice

Trichloroethylene (TCE) is classified as 'carcinogenic to humans' by the US EPA and IARC. Although it has been hypothesized that peroxisome proliferator-activated receptor alpha (PPARα) activation is a crucial event in liver carcinogenesis in rodents, uncertainties remain regarding the association between the extent of oxidative metabolism, PPARα activation, and toxicity/carcinogenesis pathways. This study investigated oxidative TCE metabolism in mouse liver and kidney in the context of PPARα status (wild-type, Ppara-null, and humanized-PPARA). Male and female mice were treated with TCE (400 mg/kg/day) by oral gavage for 4 weeks. Quantification of TCE and metabolites was performed. In addition, organ-to-body weight ratio, cellular proliferation, expression of peroxisome proliferator marker genes (Ppara, Acox1, and Cyp4a10), steatosis, and oxidative stress were evaluated. We found that there are significant differences in TCE metabolism across different genotypes, and that liver levels of TCA are associated with liver enlargement and oxidative stress regardless of PPARα status. In addition, we conducted a toxicokinetics study of TCE metabolism where concentration-time profiles of TCE and TCA were followed for up to 12 hrs in liver and kidney. We found that the difference in liver levels of TCA by genotype observed from the sub-chronic study is replicated at the toxicokinetic study and that levels of TCA appear to have longer half-life compared to levels of TCE, suggesting entero-hepatic recirculation. In conclusion, TCA may be associated with oxidative stress and liver enlargement through PPARα-independent pathway, which supports the view that PPARα activation is not the only mode of action for TCE hepatocarcinogenesis.

LAY ABSTRACT

A study of liver toxicity of a ubiquitous environmental chemical trichloroethylene

Both US Environmental Protection Agency and World Health Organization's International Agency for Research on Cancer reached a conclusion that trichloroethylene (TCE), a chemical that is widely used in a number of industrial and consumer applications such as dry cleaning, is a known carcinogen. This conclusion was based on the strength of human data showing that kidney cancer is associated with exposures to this agent. However, animal studies show that liver is the target tissue for TCE-induced carcinogenesis. Investigations into the mechanisms of why TCE may be carcinogenic have identified several important areas. First, it is how TCE is converted in the body into other compounds that are more harmful. Second, how TCE may act on proteins in cells to activate the molecular cascades that may lead to cancer. In this study, we aimed at addressing the possible relationship between these two. We used a mouse that was genetically engineered to lack the mouse version, or to have a human version, of the protein called PPAR which is one of the important triggers of the molecular events and we studied how TCE is converted to other toxic molecules. Surprisingly, we found that in mice that lack PPAR or express human version of this protein convert TCE in a different way. This information is important because in the past, scientists considered these important areas separately, but our work begins to lay a foundation for how we can understand the relationship between the two.

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TECHNICAL ABSTRACT

The Effects of Genetic Variability on the Shape of a Dose-Response Curve: 2,3,7,8 Tetrachlorodibenzo-ρ-dioxin (TCDD) induced Suppression of CD40L-activated Human Primary B Cells

Recently, the National Research Council (NRC) hypothesized that traditional nonlinear dose-response curve for non-cancer endpoints will become linear when the genetic variability of a population is taken into consideration. This hypothesis is not adequately tested and, if correct, will have broad implications for risk assessment. The purpose of this project is to close this knowledge gap by determining the shape of the dose response curve of TCDD-induced suppression of primary B-cell activation in a population of human donors. We hypothesize that incorporation of population variability will not linearize the dose-response curve as suggested by the NRC. This hypothesis will be tested via well-established TCDD-elicited suppression of immunoglobulin M (IgM) secretion from CD40L activated human B cells. Primary human B cells will be isolated from 50 unique human donors and exposed to increasing levels of TCDD (0.0001 - 30 nM). The number of IgM secreting B cells and amount of IgM secreted will be assessed following exposure. Preliminary results indicate that TCDD has a significant suppressive effect on the number of IgM secreting B cells at the top TCDD concentrations (1, 10, and 30 nM) ($p < 0.05$). Moreover, these results suggest the dose-response better fits a non-linear, sigmoidal statistical model as opposed to a linear model based on Akaike Index Criteria (AIC). Results also suggest that reactive oxygen scavengers can modulate TCDD-induced changes in IgM secretion. Taken together, the preliminary results support our hypothesis. Further analysis of unique donors will assess the effects of genetic variability on the dose-response curve.

LAY ABSTRACT

The Effects of Genetic Variability in Dose-Response Relationships

Traditional non-cancer endpoint dose-response curves result in an S-shaped/sigmoidal dose to response relationship. Recently, the National Research Council (NRC) suggested that the traditional nonlinear dose-response relationships for non-cancer endpoints would become linear when the diversity of a population is considered. This hypothesis has not been adequately tested and, if correct, will have broad implications in the risk assessment of toxic, non-cancer causing physiological outcomes. As dose-response relationships are rarely assessed from a population standpoint, the purpose of this project is to generate data to close this knowledge gap by determining the shape of the dose response curve of a population of human donors. We hypothesize that incorporation of population variability will result in an S-shaped/sigmoidal dose-response relationship. The hypothesis will be tested by comparing the relationship between a dose of 2,3,7,8 Tetrachlorodibenzo-ρ-dioxin (TCDD) dose and immune suppression activity across multiple donors. Preliminary results indicate that the top doses of TCDD resulted in a statistically significant reduction in immune cell function. Statistical modeling suggests that, when all current donors are taken into consideration, the dose-response relationship remains S-shaped/sigmoidal. Thus, while preliminary results currently support our hypothesis, including more donors will better assess the effects of genetic variability on a dose-response relationship for non-cancer outcomes.

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TECHNICAL ABSTRACT

Reactive oxygen species generation in interluekin-6 release stimulated by trichloroethylene metabolite S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) in human placental cells.

Trichloroethylene (TCE) is a common environmental pollutant associated with adverse reproductive outcomes in humans. TCE toxicity is primarily through its biotransformation to toxic metabolites, including S-(1,2-dichlorovinyl)-L-cysteine (DCVC). Although the placenta is a highly metabolic organ, TCE metabolism and toxicity in the placenta remains unknown. The present project is part of the PRoTECT Center's investigation of environmental contaminant links to adverse birth outcomes. Human extravillous trophoblast cells (HTR-8/SVneo) were exposed to 5-50 μM DCVC. We found that exposure to 10 and 20 μM DCVC decreased ATP levels ($p<0.05$) and mitochondrial membrane potential ($p<0.05$), indicating that DCVC induces mitochondrial dysfunction in HTR-8/Svneo cells. Additionally, exposure to 10 and 20 μM DCVC increased oxidant species production ($p<0.05$). We also found that 10 and 20 μM DCVC decreased glutathione and increased mRNA expression of the redox-sensitive genes glutaredoxin 2 and thioredoxin reductase 1 ($p<0.05$). Moreover, 10 and 20 μM DCVC increased mRNA expression and release of IL-6, and these responses were inhibited by with the antioxidant (±)α-tocopherol (50 μM) and deferoxamine (1 mM), which can act as an antioxidant by chelating intracellular iron. The results with antioxidants suggest that DCVC-stimulated IL-6 release in HTR-8/SVneo cells is dependent on reactive species. Treatment with 1 mM aminooxyacetic acid (AOAA), a cysteine conjugated β-lyase inhibitor, blocked DCVC-stimulated IL-6 release ($p<0.05$), suggesting that DCVC activation of this response is dependent on its bioactivation by β-lyase. Because abnormal activation of proinflammatory cytokines can lead to poor placentation, these findings suggest that exposure to TCE during pregnancy may contribute to increased risk for adverse pregnancy outcomes.

LAY ABSTRACT

Reactive oxygen species generation in interluekin-6 release stimulated by trichloroethylene metabolite S-(1, 2-dichlorovinyl)-L-cysteine (DCVC) in human placental cells.

Exposure to Trichloroethylene (TCE), a common environmental pollutant, has been associated with complications of pregnancy humans. In the body, TCE is enzymatically activated to the metabolite S-(1,2-dichlorovinyl)-L-cysteine (DCVC). Much like the kidney, the placenta is a highly metabolic organ where reactive chemical species and inflammation can lead to adverse health consequences. However, little is known about TCE metabolism and toxicity during pregnancy. We investigated the effects of the TCE metabolite DCVC on human placental cells. Cells were exposed to concentrations of DCVC similar to or lower than concentrations used in previous cell culture experiments with kidney cells. By measuring changes in mitochondrial cellular energy production (ATP) and mitochondrial function (membrane potential), we found that DCVC significantly impaired mitochondrial function. We also found that DCVC significantly increased production of free radicals, which are potentially hazardous chemicals to cells. DCVC significantly decreased glutathione, which serves as an important detoxifier of free radicals and other reactive chemicals in the cell, and increased expression of genes for enzymes that act as part of the antioxidant response to protect cells from reactive chemical species, glutaredoxin 2 and thioredoxin reductase 1. Moreover, DCVC increased expression and release of the pro-inflammatory cytokine IL-6, and this effect was reversed by exposure with antioxidant treatments (Vitamin E or deferoxamine). Additionally, an inhibitor of β-lyase, an the enzyme that increases toxicity of DCVC through further metabolism, likewise blocked the DCVC-stimulated IL-6 release. These results indicate that in these placental cells, free radicals mediate DCVC-stimulated release of IL-6 by a DCVC metabolite. These studies suggest that production of free radicals leading to abnormal secretion of IL-6 by DCVC has the potential to disrupt normal trophoblast function and may lead to improper placentation.

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TECHNICAL ABSTRACT

Detection of Protein Adducts from 4-Chlorobiphenyl (PCB3) by Alkaline Permethylation Method

In 2013 IARC classified Polychlorinated Biphenyls (PCBs) as carcinogenic to humans, with the statement that different mechanisms are involved. PCBs with lower (1-4) number of chlorines are semivolatile and have been found in urban air and indoor air of older buildings. Lower chlorinated congeners are prone to be bioactivated to electrophiles which may bind covalently to macromolecules. However, very little is known about cellular target proteins and the reactive PCB intermediates. The study was to establish a method to measure PCB adducts with cysteine residues and to identify the reactive intermediates involved in adduct formation. Several approaches, such as strong acid hydrolysis, mild acid hydrolysis, and the MT (methanesulfonic acid and trifluoroacetic anhydride) method failed to provide usable results. Finally an alkaline permethylation and GC/MS-based method was examined. Alkaline permethylation is used to convert protein-S adducts with PCBs to stable and extractable PCB derivatives with methylated hydroxide and thiol groups. The methylated PCB products can be used for quantification. Reaction conditions were modified to improve the permethylation efficiency, including reaction temperature, time, and NaOH strength. The improved conditions of alkaline permethylation include incubation with 6 N NaOH at 100 ℃ for 6 hours. With this approach treatment of PCB3-quinone adducted albumin resulted in extractable derivatives with 1-2 methylthio groups indicating binding of PCB3-quinone with 1 or 2 cysteine residues of albumin. Experiments are under way to test this method with liver microsomes for bioactivation of PCB3 and adduct formation in vitro. (Supported by NIEHS P42 ES013661).

LAY ABSTRACT

Building up the Method to Detect Proteins Binding from PCB

In 2013, the International Agency for Research on Cancer (IARC) classified PCBs as compounds to cause cancers in humans. PCBs have been found in urban air and indoor air of older buildings. PCBs with less chlorines can be changed into intermediate products which may bind to DNA or proteins. However, very little is known about cellular target proteins and the reactive PCB intermediates. Our study was to establish a method to measure PCB binding to proteins and to identify the intermediates involved. Several methods were tried, but failed to provide usable results. The alkaline permethylation (AP) method is used to convert PCBs, which are binding to proteins, to stable compounds. These stable compounds can be used to identify and quantitatively measure PCB intermediates. Mass spectrometry (MS) was applied to measure these stable compounds from AP method. Reaction conditions of AP method were modified to improve the efficiency. The improved conditions of AP method include incubation with 6 N NaOH at 100 ℃ for 6 hours. The results from AP method showed that one PCB intermediate binds to 1 or 2 cysteine amino acid of proteins. The method is still under way to test with samples. (Supported by NIEHS P42 ES013661).

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TECHNICAL ABSTRACT

Cytochrome c Adducts by Quinoid Metabolites of Polychlorinated Biphenyls (PCBs)

PCBs were widely used as industrial chemicals and are ubiquitous and persistent human and environmental contaminants. Although PCBs are human carcinogens, they are still in use in closed applications and as byproducts in pigment productions. Lower chlorinated biphenyls can be metabolized to dihydroxy metabolites and further to quinones. Quinoid metabolites may form adducts at nucleophilic sites of proteins. Even though cytochrome c does not have free sufhydryl groups, we hypothesized that the PCB-quinones covalently bind to amino groups of cytochrome c. LC-MS was used to detect adducts of cytochrome c with selected PCB-quinones in vitro. SDS PAGE gel electrophoresis followed by NBT staining was employed to separate the adducted proteins. Trypsin digestion and LC-tandem MS were applied to identify the binding sites on cytochrome c. SYBYL-X was used to simulate the conformation change of cytochrome c after binding with PCB3-para-Q. We found that more than one molecule of PCB-quinone can bind to one molecule of cytochrome c. In addition, cross-linking of cytochrome c was observed on the SDS PAGE gel. Different conditions, such as pH, incubation time and concentrations of PCB quinones, influence the formation of cross links. Lysine (K27, K39, K54/56, K73/74) and glutamic acid (E61, E62) were identified as binding sites by LC-tandem MS. Software simulation showed conformation changes of adducted cytochrome c. These preliminary data provide evidence that covalent binding of PCB quinone metabolites to cytochrome c may be included among the toxic effects of PCBs. (Supported by NIEHS Superfund Program P42 ES013661.)

LAY ABSTRACT

Identification of Cytochrome c Binding with Intermediate Products of PCBs

PCBs were widely used as industrial chemicals, and continuously exist in human and environment. Although PCBs cause cancers in human, they are still in use in closed applications and as byproducts in pigment productions. PCBs with less chlorines can be changed into intermediate products, known as metabolites, by human body. Some of the metabolites may bind to proteins. Even though cytochrome c, the model protein we chose, does not have the most reactive amino acid, we thought PCB metabolites bind to cytochrome c with other amino acids. If the binding happens, the molecular weight of cytochrome c will increase. Mass spectrometry (MS) was used to measure the molecular weight increase. Enzyme was applied to digest cytochrome c into peptides. With the digested peptides, MS was used to identify the amino acid binding sites on cytochrome c. Software SYBYL-X was applied to estimate the changes of cytochrome c after binding. We found that more than one molecule of PCB metabolites can bind to one molecule of cytochrome c. In addition, cytochrome c polymers were observed after binding. Different conditions, such as pH, incubation time and concentrations of intermediate products, influenced the formation of cytochrome c polymers. Lysine (K27, K39, K54/56, K73/74) and glutamic acid (E61, E62) were identified as binding sites. Software simulation showed changes of cytochrome c after binding with PCB metabolites. These data provide evidence that the formation of binding on cytochrome c may be included among the toxic effects of PCBs. (Supported by NIEHS Superfund Program P42 ES013661.)

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TECHNICAL ABSTRACT

Assessing Metals Exposures in the Community Surrounding the Iron King Superfund Site

The Iron King Mine and Humboldt Smelter in Northern Arizona were operational from the 1880s-1960s, resulting in millions of square feet of left-over material and by-products, and recently the US EPA has designated this area a Superfund Site. Potential metals exposure to the surrounding communities has come into question, and in response, we conducted a multi-media exposure assessment. We collected biological samples from children ages 1-11 living within 5 miles of the Site, along with environmental samples at their home. Samples were analyzed for arsenic, beryllium, aluminum, nickel, lead, cadmium, and chromium via ICP-MS. A set of questionnaires were also administered to address the child(ren)'s potential exposure to metals. We found that arsenic levels in urine were above that of the general United States population. Arsenic in tap water was above the Maximum Contaminant Level (MCL) for drinking water and levels in the soil and house dust were above the Arizona Department of Environmental Quality's Soil Remediation Level (SRL). Arsenic in the children's toenails was significantly correlated with both soil and dust, and the arsenic in urine was significantly correlated with water and dust. Individual and overall study results were sent back to the participants. To ensure our study report-back materials were tailored to environmental health interests of participants and community members, we involved the participants as well as community and government stakeholders in developing these materials. We are currently conducting dust transport modeling to better understand the potential for arsenic transport from the Site to homes in nearby communities. This study has the potential to inform the development of future interventions aimed at decreasing arsenic levels in the homes near the Iron King Mine and Humboldt Smelter Superfund Site.

LAY ABSTRACT

Assessing Metals Exposures in the Community Surrounding the Iron King Superfund Site

The Iron King Mine and Humboldt Smelter in Northern Arizona were operational from the 1880s-1960s, resulting in millions of square feet of left-over material and by-products, and recently the US EPA has designated this area a Superfund Site. Potential metals exposure to the surrounding communities has come into question, and in response, we conducted a multi-media exposure assessment. We collected biological samples from children ages 1-11 living within 5 miles of the Site, along with environmental samples at their home. Samples were analyzed for arsenic, beryllium, aluminum, nickel, lead, cadmium, and chromium via ICP-MS. A set of questionnaires were also administered to address the child(ren)'s potential exposure to metals. We found that arsenic levels in urine were above that of the general United States population. Arsenic in tap water was above the Maximum Contaminant Level (MCL) for drinking water and levels in the soil and house dust were above the Arizona Department of Environmental Quality's Soil Remediation Level (SRL). Arsenic in the children's toenails was significantly correlated with both soil and dust, and the arsenic in urine was significantly correlated with water and dust. Individual and overall study results were sent back to the participants. To ensure our study report-back materials were tailored to environmental health interests of participants and community members, we involved the participants as well as community and government stakeholders in developing these materials. We are currently conducting dust transport modeling to better understand the potential for arsenic transport from the Site to homes in nearby communities. This study has the potential to inform the development of future interventions aimed at decreasing arsenic levels in the homes near the Iron King Mine and Humboldt Smelter Superfund Site.

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TECHNICAL ABSTRACT

Novel applications of a high-capacity cellular screening assay for respiratory toxicology

The cells that line the airways, collectively termed the airway epithelium, provide the first line of defense against inhaled particulates, pathogens, and toxicants. The airway epithelium traps and removes inhaled insults out of the respiratory tract and provides a barrier to the underlying tissue. It is well accepted that certain inhalation toxicants can lead to obstructive lung diseases, in part by compromising the airway epithelium. Traditional cellular toxicity assays represent an end point in toxicity analysis, however, diseases commonly progress with compromised cellular function in lieu of cell death. To better assess cell-compromising toxins on airway epithelial cells, we have used the xCELLigence Real-Time Cell Analyzer (RTCA). The RTCA is a high-capacity screening device that measures cellular growth, death, and morphological changes associated with mechanisms such as cell signaling. We have used the RTCA to measure sub-cytotoxic signaling effects in human bronchial epithelial cells. Responses to cell signaling agonists important in normal airway epithelial function were measured in untreated cells and compared to responses in cells treated for 4 - 24 hrs with arsenic, nanoparticles, or electronic (e-) cigarette constituents. We found that these respiratory toxicants caused altered signaling responses compared to untreated cells. We confirmed the effects of these toxicants on airway epithelial cell signaling with low-capacity, direct measurement of intracellular signaling. In conclusion, the RTCA device offers a high-capacity approach to screening cells for toxin/toxicant-induced dysfunction independent of cell death and potentially important in the onset of chronic airway disease.

LAY ABSTRACT

Novel applications of a high-capacity cellular screening assay for respiratory toxicology

The cells that line the airways, collectively termed the airway epithelium, provide the first line of defense against inhaled particulates, pathogens, and toxicants. The airway epithelium traps and removes inhaled insults out of the respiratory tract and provides a barrier to the underlying tissue. It is well accepted that certain inhalation toxicants can lead to obstructive lung diseases, in part by compromising the airway epithelium. Traditional cellular toxicity assays represent an end point in toxicity analysis, however, diseases commonly progress with compromised cellular function in lieu of cell death. To better assess cell-compromising toxins on airway epithelial cells, we have used the xCELLigence Real-Time Cell Analyzer (RTCA). The RTCA is a high-capacity screening device that measures cellular growth, death, and morphological changes associated with mechanisms such as cell signaling. We have used the RTCA to measure sub-cytotoxic signaling effects in human bronchial epithelial cells. Responses to cell signaling agonists important in normal airway epithelial function were measured in untreated cells and compared to responses in cells treated for 4 - 24 hrs with arsenic, nanoparticles, or electronic (e-) cigarette constituents. We found that these respiratory toxicants caused altered signaling responses compared to untreated cells. We confirmed the effects of these toxicants on airway epithelial cell signaling with low-capacity, direct measurement of intracellular signaling. In conclusion, the RTCA device offers a high-capacity approach to screening cells for toxin/toxicant-induced dysfunction independent of cell death and potentially important in the onset of chronic airway disease.

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TECHNICAL ABSTRACT

PCB126 mediated disruption of hepatic metal homeostasis in mice and the role of metallothionein

Polychlorinated biphenyls (PCBs), industrial chemicals and persistent environmental pollutants, are found in rural and urban settings. Rodent studies have shown that exposure to PCB126, causes a significant disruption of hepatic metals homeostasis and an increase in metallothionein (MT), an antioxidant protein and metal carrier. The current study investigates the role of metallothionein in this phenomenon. Twenty four 129S male mice were obtained from Jackson labs (12 wild type (WT) and 12 MT knockout (KO)) and placed on a purified diet (AIN-93G) for 3 weeks to achieve hepatic metal equilibrium. Mice were then given a single injection, IP, of either soy oil or 150 umol/kg PCB126 in soy oil. The animals were sacrificed 2 weeks later and organs processed for analysis. The expression of AhR regulated genes were investigated by qRT-PCR, hepatic metals status determined by inductively coupled plasma mass spectroscopy (ICP-MS), and intracellular metals status was investigated using energy dispersive spectroscopy transmission electron microscopy (EDS-TEM). Liver tissue was also analyzed histologically. Liver weights increased with PCB126 exposure; however no difference was seen between WT and KO. Hepatic metals levels (Cu, Zn, Mn and Se) were investigated and their disruption follows a similar pattern that has been seen before. Histologically, the liver shows signs of steatosis with PCB126 exposure with the most marked change in the MT-knockout treated animals. Metallothionein has been shown to modulate metal status by its induction; this research suggests that MT may not be the sole cause of the metal disruption caused by PCB126 exposure. (P42 ES013661)

LAY ABSTRACT

Micronutrient changes in the liver after PCB126 exposure may not be linked to common metal carrier, metallothionein.

Micronutrients, like Cu, Zn, Mn, and Se, are seen to change with exposure to PCB126. The cause of this is unknown but may be linked to metal carrier proteins, in particular metallothionein. Metallothionein is a small protein that binds metals and also acts as an antioxidant. This study used mice which lack metallothionein to assess the role of it in the disruption of hepatic micronutrients. Although micronutrient levels changed with PCB126 treatment no difference was seen with/without the presence of metallothionein. However, the hepatic damage was increased in the mice lacking metallothionein. This suggests that metallothionein may not be involved in this alteration throughout the liver. Interestingly, increases in toxicity of PCB126 in animals which lack metallothionein implies that metallothionein can protect the liver against PCB126 exposure. The possibility of a protective effect of metallothionein will be investigated in future studies.

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TECHNICAL ABSTRACT

Environmentally persistent free radicals (EPFRs) degrade left ventricular function during ischemia/reperfusion injury.

EPFRs in particulate matter (PM) produce reactive oxygen species and oxidative stress. Inhalation of the EPFR DCB230 (1,2-dichlorobenzene on 5% CuO-coated silica) reduces cardiac function and produces oxidative stress in the left ventricle (LV) of rats. Since oxidative stress is a hallmark of myocardial ischemia/reperfusion injury (MI/R), we hypothesized EPFRs may increase cardiac vulnerability to MI/R. Rats were exposed to DCB230 or vehicle by inhalation (230μg max/d) for 30min/d over 7d. MI/R or sham MI/R was induced 24hrs post-exposure. Following reperfusion for 1d or 7d (1d MI/R, 7d MI/R), LV function was assessed and infarct size was measured. In vehicle-exposed rats, MI/R did not reduce stroke volume (SV), cardiac output (CO), stroke work (SW), end-diastolic volume (EDV), or end-systolic volume (ESV) after 1d reperfusion, however, end-systolic pressure (ESP) was reduced. The ESP-volume relationship (ESPVR) and preload-recruitable SW (PRSW) were elevated. 48hrs after DCB230, sham MI/R rats showed expected reductions in EDV. ESPVR was increased attempting to maintain LV function; however, PRSW was not increased. DCB230 1d MI/R rats had decreased ESP without further reductions in SV or CO compared to controls. SV and CO were reduced compared to vehicle 1d MI/R rats. Due to the decrease in EDV, DCB230 1d MI/R rats did not increase PRSW to match the LV function of vehicle 1d MI/R rats. DCB230's effects on LV function dissipated within 8d. Infarct size was not different between groups. Thus, inhalation of EPFRs transiently degrades cardiac function during MI/R, possibly linking PM and MI/R-related mortality.
Support P42ES013648,P20RR018766.

LAY ABSTRACT

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TECHNICAL ABSTRACT

Cellular biomarkers of cadmium neurobehavioral injury in Coho salmon

Cadmium (Cd) and other trace metals are potent inhibitors of olfactory function in salmonids and other fish species. However, limited behavioral and mechanistic data on metal olfactory injury has prevented the development of reliable biomarkers to assess the effects of metal exposures on salmon migrating through Superfund sites. In the current study, we examined the effects of Cd on salmon neurobehavior, and investigated a suite of molecular biomarkers encoding proteins critical in maintaining olfactory function. The suite included markers of major olfactory G-protein coupled receptors (GPCRs), a marker of neurite growth, and markers of metal induced oxidative stress. In vivo acute and sub-chronic exposures to environmentally relevant concentrations of waterborne Cd (0.2, 3, 30 and 300 ppb) were conducted using juvenile Coho salmon. Olfactory mediated behavioral analysis showed a loss of detection and responses to specific odorants following Cd exposures. A dose-dependent loss of olfactory GPCR and neurite gene expression was observed. This persisted even after a 2-week recovery period, indicating that these biomarkers reflect recent Cd exposures. By contrast, the induction of oxidative stress markers reflected a protective olfactory antioxidant response to Cd injury. Histological analysis showed that the molecular biomarkers were strongly associated with Cd-induced losses of olfactory receptor neurons within the olfactory epithelium. In summary, exposure to environmental levels of Cd relevant to aquatic Superfund sites can induce long-lasting olfactory behavioral dysfunction in salmon that is reflected by a suite of molecular biomarkers. University of Washington Superfund Research Program (ES-04696).

LAY ABSTRACT

Developing sensitive markers of cadmium-inhibition of odorant perception in Coho salmon

The ability to smell and respond to chemical cues in the environment is essential for salmon survival and reproduction. Cadmium (Cd) and other trace metals are potent inhibitors of olfaction in salmon and other fish. However, the lack of studies addressing the biochemical mechanisms of metal-induced olfactory injury has prevented the development of biomarkers to assess the effects of metal exposures on salmon migrating through Superfund sites. In the current study, we examined the effects of Cd on odorant-driven behaviors and investigated a suite of molecular biomarkers critical in maintaining olfactory function. The suite included biomarkers of key structures within the olfactory system, including odorant receptors (ORs), immature neurons, and also olfactory cell antioxidant defenses against metals. Behavioral analyses in Cd-exposed salmon showed a reduced ability to detect and respond to prototypical chemical odorants. A dose-dependent loss of gene expression of ORs and the immature neurons occurred, and persisted after a two week recovery period, indicating that these biomarkers could reflect recent Cd exposures. By contrast, the induction of cellular defense (i.e. oxidative stress) markers reflected a protective antioxidant response to Cd injury. Histological examination revealed that the molecular biomarkers were strongly associated with Cd-induced losses of odor sensing neurons. In summary, exposure to environmental levels of Cd relevant to aquatic Superfund sites can induce long-lasting dysfunction in the salmon's ability to smell that is reflected by a suite of molecular biomarkers. University of Washington Superfund Research Program (ES-04696).

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TECHNICAL ABSTRACT

Chemoproteomic Profiling of Environmental Electrophiles Reveals Toxicity Through Altered Cellular Metabolism

The large numbers of chemicals we are exposed to necessitates a better understanding of their interactions within complex biological systems to more accurately predict toxicities. Though many chemicals have incomplete toxic mechanisms due to unknown direct target interactions, reactive electrophilic chemicals that can interact covalently with metabolic enzymes, causing widespread alterations in biochemical networks are a particular concern. Needed are in depth toxicological assessments that provide the direct protein targets of reactive chemicals in complex systems and reveal the resulting biochemical effects of this interaction. To address this, we have developed a chemoproteomic technology called reactivity-based protein profiling (RBPP) that employs bioorthogonal chemical probes that bind to hyperreactive residues such as cysteines within complex proteomes. Using RBPP, we first identify the direct and specific protein targets of reactive environmental chemicals in vivo, then examine the consequences of target engagement using functional metabolomics to reveal downstream metabolic changes. In this study we used RBPP to screen highly used environmental electrophiles and then identified novel targets of the highly reactive yet widely used fungicide chlorothalonil in vivo in mice. A bioorthogonal chlorothalonil probe revealed targets that include a network of hyperreactive cysteine-containing metabolic enzymes spanning multiple conserved metabolic networks. Functional metabolomic analysis revealed dramatic alterations in critical lipid and central carbon metabolic pathways, suggesting broad toxicity through previously undescribed mechanisms. Using techniques like RBPP to map proteome-wide interactions and biochemical effects of reactive chemicals is critical for prioritizing action on existing toxicants and for developing safer chemicals towards minimizing exposure-related health effects.

LAY ABSTRACT

A New Profiling Method For Reactive Environmental Chemicals Reveals Direct Targets and Toxic Effects

The large numbers of chemicals we are exposed to necessitates a better understanding of their interactions within complex biological systems to more accurately predict toxic effects. Though we do not completely understand the way many chemicals cause toxicity, highly reactive chemicals that can interact with enzymes and affect normal metabolism are a particular concern. We need in depth tests that directly identify the protein targets of these reactive chemicals in a complex system and then reveal the effects on metabolism from the chemical-protein interaction. To address this, we have developed a technology called reactivity-based protein profiling (RBPP) that uses chemical probes that bind to chemical-sensitive amino acids in a complex system. Using RBPP, we first identify the direct protein targets of reactive environmental chemicals, then examine changes in metabolites to assess the consequences of the chemical-protein interaction. In this study we used RBPP to screen highly used environmental chemicals and then identified targets of the highly reactive yet widely used fungicide chlorothalonil in vivo in mice. Our chemical probes revealed targets that include a network of chemical-sensitive metabolic enzymes involved in key pathways in energy metabolism. Comprehensive metabolite analysis revealed dramatic alterations in critical metabolic pathways, suggesting previously undescribed toxic effects of chlorothalonil. Using techniques like RBPP to map chemical-protein interactions and metabolic effects of reactive chemicals in complex systems is critical for prioritizing action on existing toxicants and for developing safer chemicals to minimize health effects from exposure to chemicals.

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TECHNICAL ABSTRACT

Limited impact of point-of-use filters on arsenic exposure in the Folate and Creatinine Trial (FACT)

The efficacy of household-level arsenic (As) removal filters to treat tubewell water in Bangladesh remains unclear. The Folate and Creatine Trial (FACT) was a double-blind, placebo controlled, randomized trial; all participants were chronically exposed to water arsenic (wAs) between 50-1182ug/L. Upon enrollment, all were given point-of-use water filters (READ-F filters) to use for all cooking and drinking. Among participants who continued to use their same well one year post-trial, untreated wAs remained within 10% of the baseline concentration for 157 of 188. Of the 31 remaining filters purportedly still used 12 months after the trial ended, there were two cases where treated wAs >50ug/L and in three cases 10ug/L< wAs <50ug/L. Although urinary As concentrations (uAs) dropped from an average of 157.1±158.1ug/L at baseline to 91.3±89.5ug/L 6 weeks into the trial, uAs gradually rose to 171.9±161.1ug/L by week 24. This indicates a decline in filter use that is consistent with complaints from 375 out of 557 participants who, 12 months after the trail ended, responded that they had stopped using filters after the study because filters became inconvenient as water flow slowed. In contrast, participants who switched to a low-As well had lower uAs compared to filter-users; uAs among well-switchers declined to 49.4±29.9ug/L (n=9) 12 months after the trial. Point-of-use wAs filters successfully removed As from contaminated tubewells early in the FACT trial, however, filter inserts require maintenance and are costly. Point-of-use filters can temporarily reduce arsenic exposure in Bangladesh but do not appear to be a viable long-term option.

LAY ABSTRACT

Characterizing the impact of filter use habits on water arsenic exposure

The long-term effectiveness of filters to remove arsenic from water (wAs) is unknown, yet they are used to try to reduce exposure. The Folate and Creatine Trial (FACT) is a randomized controlled trial in Bangladesh. Because all FACT participants were consuming high wAs, they were provided with an arsenic-removal water filter upon enrollment. Participants were instructed to use it for all cooking and drinking throughout the 6 month trial. This intervention and a follow-up visit one year after the trial ended provided the opportunity to examine whether filters offer a practical and sustainable strategy to lower arsenic exposure. Tubewell wAs tested both at FACT baseline and at the revisit were significantly correlated, indicating unfiltered wAs is stable over time. During the trial, the filters successfully lowered wAs; however, out of 31 filters tested at revisit, 6% had wAs>50ug/L (n=2) and 10% had wAs>10ug/L and <50ug/L (n=3), exceeding wAs guidelines for Bangladesh and WHO, respectively. One year after the trial ended, 95% reported they had stopped using the filter. Filters filtered water more slowly over time, and the filter inserts require maintenance, thus becoming inconvenient. Self-reported “always-filter-users” did not have lower urinary arsenic compared to self-reported “never-filter-users”, suggesting non-compliance. “Never-filter-users” who reported switching to a nearby low-arsenic well had significantly lower urinary arsenic compared to participants who reported always using their filter. Thus, efforts toward prolonged filter use did not lower arsenic exposure, whereas well-switching did. Point-of-use-filters should not be considered as a sustainable arsenic mitigation option in Bangladesh.

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TECHNICAL ABSTRACT

The Inhibition of Human Steroid Sulfotransferases hSULT1E1 and hSULT2A1 by Hydroxylated and Sulfated Metabolites of Polychlorinated Biphenyls

Polychlorinated Biphenyls (PCBs) are persistent and pervasive environmental toxicants. We are currently examining the interactions of human sulfotransferases hSULT1E1 and hSULT2A1 with hydroxylated and sulfated metabolites of PCBs (OH-PCBs and PCB sulfates, respectively) that are derived from those PCB congeners most commonly found in air samples. These sulfotransferases catalyze the sulfation (inactivation) of estrogens and androgens, and we hypothesize that these OH-PCBs and PCB sulfates differentially inhibit hSULT1E1 and hSULT2A1. Inhibition of purified recombinant hSULT1E1 (7.0 nM estradiol as substrate) and hSULT2A1 (1.0 uM DHEA as substrate) was determined. 4'-PCB 3 sulfate and 4-PCB 11 sulfate did not show inhibition of hSULT1E1 up to a concentration of 10 uM, whereas 4'-PCB 25 sulfate displayed an IC50 of 233 +/- 56 nM. The IC50 values for inhibition of hSULT1E1 by 4-OH-PCB 3, 4-OH-PCB 11, and 4'-OH-PCB 25 were: 1300 +/- 550 nM, 7.2 +/- 1.5 nM, and 7.3 +/- 2.3 nM, respectively. The IC50 value for inhibition of hSULT2A1 by 4'-PCB 25 sulfate was 56 +/- 50 uM. 4'-PCB 3 sulfate exhibited minimal inhibition, and 4-PCB 11 sulfate showed no inhibition at concentrations up to 100 uM. The IC50 values for inhibition of hSULT2A1 by 4'-OH-PCB 3, 4-OH-PCB 11, and 4'-OH-PCB 25 were 12 +/- 4.8 uM, 23 +/- 3.9 uM, and 4.9 +/- 0.9 uM, respectively. Such interactions with hSULT1E1 and hSULT2A1 may have implications for alterations in steroid hormone signaling, and this is a subject for further investigation. [Supported by NIH P42 ES013661 and by R25 GM058939]

LAY ABSTRACT

The Inhibition of Human Steroid Sulfotransferases hSULT1E1 and hSULT2A1 by Hydroxylated and Sulfated Metabolites of Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are persistent environmental toxins that have associations with multiple adverse health effects. While sulfation is involved in the metabolism and detoxication of many environmental toxins, such as PCBs, it is also an important biological process that results in the normal cellular inactivation of steroid hormones, such as androgens and estrogens. Sulfation therefore regulates the cellular concentrations of the active hormones. Sulfotransferases are enzymes that catalyze the process of sulfation. If the sulfotransferases are blocked from carrying out sulfation, this could lead to abnormally high concentrations of active steroid hormones in specific locations resulting in a disruption of the balance between active and inactive hormones at those sites. The goal of our project is to determine if hydroxylated and sulfated metabolites of those PCBs that are commonly found in outdoor and indoor air samples are capable of inhibiting the activity of the estrogen and androgen sulfotransferases. Our preliminary studies have shown that the hydroxylated PCB metabolites examined inhibit these enzymes, while the sulfated PCB metabolites are much less effective at this inhibition. Such interactions with these sulfotransferases may have implications for alterations in steroid hormone signaling, and this is a subject for further investigation. [Supported by NIH P42 ES013661 and R25 GM058939]

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TECHNICAL ABSTRACT

In Utero Exposure to Cookstove Emissions: Epigenome-Wide Associations and Immunological Changes in Cord Blood

There is increasing evidence that indoor air pollution exposure during pregnancy is a risk factor for poor birth outcomes, immunological disruption and low cognitive performance. Chemicals present in cookstove emissions have the ability to cross the placental barrier exposing the developing fetus. DNA methylation is an important mechanism for fetal programming during development and demonstrated to be sensitive to environmental insults. This study aimed to evaluate the association of cookstove emissions exposure during pregnancy, immunological changes and differences in DNA methylation in cord blood. Cord blood was collected from 44 infants recruited in a prospective birth cohort in Bangladesh and analyzed for DNA methylation using the Illumina Infinium Methylation450K array. Prenatal exposure to cookstove emissions was evaluated from maternal questionnaires by self-reported time spent cooking over an open fire. White blood cell composition was estimated using a statistical method to infer cell mixture from DNA methylation profiles. We observed a 1.81% mean decrease in monocytes with increasing prenatal exposure to cookstove emissions (P=0.002). Furthermore, we identified one differentially methylated CpG site (cg20531392) found in a CpG island of the DUSP5 gene after adjusting for multiple comparisons (q-value= 0.043). Overexpression of DUSP5 gene has been shown to suppress the growth of several types of human cancer cells and hypothesized to act through a p53-dependent mechanism. This work supports the hypothesis that prenatal exposure to cookstove emissions may affect the developing immune system and may influence fetal programming. Future research should investigate if these changes are associated with adverse health outcomes.

LAY ABSTRACT

Exposure to cookstove emissions during pregnancy associations with epigenetic changes and immune function

Nearly three billion people use solid fuels like crop residue and wood to meet their household energy needs. If a woman is pregnant and using solid fuels for cooking, her fetus can be exposed to chemicals emitted from burning which can lead to adverse health outcomes in children including low birthweight and increased risk of respiratory illnesses. We hypothesize that cookstove emissions might also affect the epigenome, or how genes are expressed. This is important because the epigenome is largely established during fetal development and controls which genes get turned on or off. We investigated the relationship between cookstove emissions that occurred during pregnancy and DNA methylation, a specific type of epigenetic change, by looking at DNA in cord blood collected from 44 newborns in Bangladesh. Using epigenetic tools we also evaluated how cookstove emissions influenced white blood cells. We observed that infants who were born to women who spent the most time cooking over an open fire had a small decrease of 1.8% in monocytes which is a white blood cell that removes debris and microorganisms from our bodies. We also detected an epigenetic change in the DUSP5 gene. This gene has been shown to act together with another important gene, p53 controlling cell development and inhibiting tumor growth in cancer cells. This work demonstrates that exposure to cookstove emissions during pregnancy can alter the immune response and modify epigenetic events that occur during development. Future research should address if these changes are harmful to human health.

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TECHNICAL ABSTRACT

Computational high throughput quantitative analysis of dose-dependent histological features

Manual quantitation of stained features can be onerous and subjective, yet valuable when integrated with complementary omic data. High resolution slide digitization facilitates the development of computational alternatives. We developed a MATLAB-based quantitative histological analysis tool (QuHAnT) for the high-throughput assessment of distinguishable histological features. Validation was demonstrated by comparison to manual quantitation using liver sections from mice orally gavaged with sesame oil vehicle or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; 0.001- 30 μg/kg) every 4 days for 28 days which elicits hepatic steatosis with mild fibrosis. A quality control module reduced the number of quantifiable Oil Red O (ORO) images from 3,123 to 2,756. ORO staining was increased ($p < 0.05$) at 10 and 30 μg/kg TCDD with a strong correlation between manual and computational volume densities (Vv), but a 10-fold greater dynamic range with QuHAnT. Additionally, QuHAnT determined the size of each ORO vacuole which could not be accurately measured by visual examination or manual point counting. Dose-response modeling of manually counted ORO stained features estimated ED50, BMD, and BMDL values of 10, 0.26, and 0.12 μg/kg TCDD, respectively, and 27, 1.40, and 1.01 μg/kg TCDD, respectively, using QuHAnT which more closely reflected visual assessment and lipid associated differential gene expression. QuHAnT quantitation of PicroSirius Red (PSR) staining demonstrated superior detection of collagen deposition due to the ability to consider all images within each section. In summary, QuHAnT reduced analysis time and facilitated a more comprehensive assessment of features of interest with improved accuracy and sensitivity. Funded by SRP P42ES04911.

LAY ABSTRACT

Computational high throughput quantitative analysis of dose-dependent histological features.

In the assessment of chemical toxicity microscopic evaluation of organs is critical in identifying toxic responses. The quantitative assessment of these lesions assists in determining safety levels. To date, quantitative assessment involved manual counting, but recent advances in slide digitization has made computational approaches a viable alternative. We developed a computational tool (Quantitative Histological Analysis Tool (QuHAnT) which rapidly quantifies the feature of interest on a large numbers of slides. To demonstrate that QuHAnT is consistent with current standards we used mouse livers treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which causes lipid accumulation and fibrosis. Manual quantitation of lipid droplets was compared to QuHAnT for 2,756 images and showed a strong correlation. QuHAnT was also able to measure an dose-dependent increase in the size of individual lipid droplets, something not easily done using a manual approach. We performed dose response modeling to determine doses at which exposure begins to elicit a response (BMD, BMDL) and reaches 50% of maximal response (ED50) using both datasets. QuHAnT values were most consistent with gene expression responses. Similar validation of fibrosis quantitation also demonstrated superior performance of our computational tool because it was able to consider all of the available images. Overall, QuHAnT reduces analysis time and facilitates the comprehensive assessment of features with improved accuracy and sensitivity. Funded by SRP P42ES04911.

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TECHNICAL ABSTRACT

Comparative Assessment of Dose-Dependent TCDD-elicited Hepatic Gene Expression in Mice.

Differential gene expression plays a critical role in the mechanism of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Reductions in sequencing cost, have made RNA sequencing the preferred technology to assess global gene expression with microarrays, although still common, becoming obsolete. We compared the hepatic transcriptome of C57BL/6 mice following gavage with sesame oil vehicle, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, or 30 ug/kg TCDD every 4 days for 28 days using Illumina HiSeq RNA-Sequencing (RNA-Seq) and Agilent 4x44K microarrays. RNA-Seq and microarray analysis identified a total of 16,403 and 18,247 genes, respectively, that were expressed in the liver. Analysis for differentially expressed genes (DEGs) varied dramatically depending on the P1(t) cut-off used for RNA-Seq data while microarray results varied more based on the fold change criteria, although responses strongly correlated. Verification by WaferGen SmartChip QRTPCR revealed that RNA-Seq had a false discovery rate of 24% compared to 54% for microarray analysis. Dose-response modeling of RNA-Seq and microarray data demonstrated similar ED50 and BMD estimates for common DEGs. Interestingly, three distinct ED50 peaks were identified suggesting a step-wise dose-dependent activation of lipid metabolism, immune system response, and collagen deposition. Although the results were comparable between the two platforms, RNA-Seq provided more qualitative and quantitative data compared to microarrays. Moreover, both RNA-Seq and microarray analyses demonstrated that TCDD elicited dose-dependent differential gene expression consistent with the progression of steatosis to steatohepatitis with fibrosis. Funded by SRP P42ES04911.

LAY ABSTRACT

RNASeq, Microarray and WaferGen QRTPCR Gene Expression Analysis in the Liver Following TCDD Treatment

Examining gene expression is essential for determining how chemicals may cause toxicity. Several technologies can be used to examine gene expression changes including RNA-Sequencing (RNA-Seq), microarrays and QRTPCR. Although microarrays have been the most popular approach, RNA-Seq is emerging as the preferred technology. To examine gene expression changes we used these technologies to look at the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the livers of mice. Statistical analysis identified 1,270 and 901 genes to be changed by TCDD exposure using RNA-Seq and microarray, respectively. Genes that were determined to be different in their responses (e.g. up-regulation by RNA-Seq and down-regulation by microarray) were evaluated using the 'gold standard' for assessment of gene expression levels (QRTPCR) demonstrating that RNA-Seq is the more reliable technology. We also modeled the responses to identify the doses at which gene expression changes compared to control (Benchmark Dose; BMD) or reach 50% of maximal response (ED50). The values closely reflected the changes seen such as altered lipid metabolism, immune responses, and fibrosis. Overall, we show that RNA-Seq outperforms microarray analyses, and that the changes in gene expression in the liver of mice exposed to TCDD reflect the phenotypes observed.

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TECHNICAL ABSTRACT

Cytotoxicity following naphthalene exposure in microsomal epoxide hydrolase deficient mice

Naphthalene (NA) is a ubiquitous pollutant to which humans are widely exposed. 1,2-Dihydro-1,2 dihydroxynaphthalene (diol) is a major metabolite of NA generated by microsomal epoxide hydrolase (mEH). To investigate the role of the NA dihydrodiol and subsequent metabolites (ie the 1,2-quinone) in cytotoxicity, we exposed both male and female wild type (WT) and mEH null mice (KO) to NA by inhalation (5, 10, 20 ppm; 4 hrs). NA dihydrodiol was ablated in the KO mice. High-resolution histopathology was used to measure airway injury. Cytotoxicity varied by airway generation and exposure concentration. Swollen/vacuolated airway epithelial cells were observed in the upper and lower airways of all mice at 20 ppm, with significantly higher damage in KO for lower airways. There was significantly more damage in upper airways of WT mice than KO at 5 and 10 ppm. There was low cytotoxicity in all lower airways at 10 and 5 ppm. Our results indicate that the apparent contribution of mEH-dependent metabolites to toxicity differs by location in the lung. For the upper airways, WT cells are more susceptible. In the lower airways, where mouse lung tumors are known to form, cells from KO mice are more susceptible to NA injury than WT. These studies suggest that either (1) metabolites generated through the mEH pathway are of minor importance in the overall toxicity and subsequent distal airway carcinogenesis from NA exposure or that (2) compensatory alterations in gene expression associated with mEH KO alter other pathways in the activation and detoxification of NA.

LAY ABSTRACT

Susceptibility to naphthalene lung injury in mice without microsomal epoxide hydrolase

Air pollution is a serious and complex public health problem. One potentially harmful air pollutant is naphthalene (NA), which causes toxic, cellular damage that can result in lung tumors in mice. Our laboratory studies the mechanisms of NA cellular damage in animals in order to better understand the possible risks of human NA exposure. We know that when humans or animals breathe NA, our lungs have mechanisms in place to process the air pollutant. Our body's enzymes break down the NA into metabolites that can either be cleared or cause damage. However, there is currently debate over which NA metabolites cause lung toxicity. Our study evaluated whether NA still had toxic effects when a major enzyme, microsomal epoxide hydrolase (mEH), was removed from mice. By eliminating the mEH enzyme, we limited which metabolites formed. We then assessed lung damage after allowing mice to breathe different concentrations of NA. Higher concentrations of NA were associated with the most lung cell damage, however damage was seen even at "safe" exposure levels. Interestingly, we found that the contribution of mEH to toxicity differed by airway location. In the lower airways, where mouse lung tumors are known to form, lung cells without mEH were more susceptible to damage from NA. This suggests that either (1) mEH metabolites are of minor importance in the overall toxicity of NA in this critical site or (2) that removing the mEH enzyme also altered other important enzyme processes that are involved in the activation and detoxification of NA.

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TECHNICAL ABSTRACT

Electrochemical Degradation of Chlorobenzene in Groundwater Using Pd- Catalytic Electro-Fenton's Reaction

In this study, a three electrode flow system is proposed for Pd-catalytic oxidation of chlorobenzene (CB) in groundwater. The system is consisted of sequentially arranged electrodes, one mixed metal oxide (MMO) anode and two MMO cathodes. Applied current is divided between cathodes to develop acidic vicinity around the first cathode. Two grams of Pd/Al2O3 is packed at the top of the first cathode to catalyze electrochemically generation of H2O2. Column experiments are conducted to investigate the system variables. Their performance for CB removal was evaluated in open flow column at room temperature. The results indicate that three electrodes system with supported Pd/Al2O3 on the surface of cathode can be used for the removal of CB pollution and their capacity does not depend on the nature of the CB concentration. Also, the three MMO electrodes provide more acidic conditions comparing two electrode systems for better oxidation. Compared with the dehalogenation with a total CB removal of 44% in 2 h with Pd/Al2O3, the CB removal reached 54.4-68.4% with Pd/Al2O3 supported with ferrous salts under the same operate condition. With the proposed treatment, the electrochemical process supported with Pd keep the degradation of CB for long time without replacement.

LAY ABSTRACT

Electrochemical Degradation of Chlorobenzene in Groundwater Using Palladium (Pd)- Catalytic Electro-Fenton's Reaction

Chlorobenzene (CB), with a chemical formula of C6H5Cl, is an aromatic organic compound. CB is a colorless, flammable liquid which is generally used in the synthesis of various pesticides, dye, and solvents. Extensive use of chlorobenzene in industry and agriculture has caused vast amounts to release into the environment. Chlorobenzene can bio-accumulate through food chain and lead to cancer, mutagenesis and damage of the nervous system. It has been identified as a priority pollutant by the US Environmental Protection Agency (EPA). In this study, a three electrode flow system is proposed for the oxidation of CB in groundwater by using an advanced oxidation process called Pd-catalytic electro-Fenton oxidation. The system consists of sequentially arranged electrodes, one mixed metal oxide (MMO) anode and two MMO cathodes. The effects of six affecting parameters (Pd presence, Fe concentration, current intensity, flow rate, electrode arrangement three electrodes vs two electrodes and time) on the CB removal efficiency were investigated. Results show that, in optimum conditions, using Pd-catalytic electro-Fenton reaction can yield up to 67% CB removal.

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TECHNICAL ABSTRACT

Alterations in the proteome in the respiratory tract in response to single and multiple exposures to naphthalene

Protein adduction is considered to be critical to the loss of cellular homeostasis associated with environmental chemicals undergoing metabolic activation. Despite considerable effort, our understanding of the key proteins mediating the pathologic consequences from protein modification by electrophiles is incomplete. This work focused on naphthalene-induced acute injury of respiratory epithelial cells and tolerance which arises after multiple toxicant doses to define the initial cellular proteomic response and later protective actions related to tolerance. Airways and nasal olfactory epithelium from mice exposed to 15 ppm NA either for 4 hrs (acute) or for 4 hrs/day x 7 days (tolerant) were used for label free protein quantitation by LC/MS/MS. Cyp2f2 and secretoglobin 1A1 are decreased dramatically in airways of mice exposed for 4 hrs, a finding consistent with the fact that P450's are localized primarily in Clara cells. A number of heat shock proteins and protein disulfide isomerases, which had previously been identified as adduct targets for reactive metabolites from several lung toxicants, were upregulated in airways but not olfactory epithelium of tolerant mice. Protein targets that are upregulated in tolerance may be key players in the pathophysiology associated with reactive metabolite protein adduction. Supported by NIEHS 04699

LAY ABSTRACT

Changes in proteins of airway lining cells in response to an inhaled cytotoxic agent

One of the major challenges in toxicology involves extrapolation of experimental results obtained in rodents to exposed human populations. In many cases human epidemiologic data are not available or are confounded by exposures to multiple chemicals. These studies are part of a long term strategy using a highly selective lung toxicant in animals to understand mechanisms and determine whether these extrapolate to exposed human populations. Studies of the toxicity of naphthalene to the respiratory system are a case in point. Naphthalene produces acute, dose dependent injury to nasal epithelial cells in both rats and mice and airway epithelial cell injury in mice when administered either by inhalation or intraperitoneal injection.

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TECHNICAL ABSTRACT

Analysis of Benzene-Induced Gene Expression in Exposed Workers by nCounter and RNA-Seq

Benzene is an established cause of leukemia and other blood disorders. In order to identify gene expression biomarkers of early effect, previously we analyzed the peripheral blood mononuclear cell (PBMC) transcriptomes of workers occupationally exposed to a range of benzene exposures (>10ppm to <1ppm) and unexposed controls. We identified a robust gene expression signature associated with high and low levels of benzene by microarray in 125 workers. We also found altered expression of additional transcripts by RNA-sequencing (RNA-Seq) in a subset (n=20) of exposed and control subjects. Here, we sought to: 1) confirm altered expression of 26 microarray signature genes using nCounter digital counting technology; and 2) to explore the possibility of detecting non-human transcripts indicative of infection in the RNA-Seq data. The nCounter assay was performed in 96 microarray subjects, randomized by age, gender and smoking status. For most genes, the Pearson correlation between the nCounter log counts and the microarray log intensities was ≥0.8, with 20 genes ≥0.9, thus validating the microarray gene expression signature. As proof-of-principle of non-human RNA detection, we used PathSeq software to subtract the non-human sequences from the RNA-Seq data of two study subjects. Re-mapping these unmapped reads revealed the presence of both viral and bacterial transcripts. We are currently examining the effect of benzene exposure on non-human transcripts. We will expand these data in a larger number of subjects in order to develop robust biomarkers of early effect of low-dose benzene. (Supported by NIEHS P42ES004705 to MTS)

LAY ABSTRACT

Detection of changes in the blood cells of people exposed to benzene

Benzene is a chemical found at Superfund sites that causes leukemia and other blood disorders. Cellular processes are driven by gene expression—the generation of RNA, and ultimately protein, from genes. Comparison of the patterns of gene expression in blood cells from people exposed to benzene with those of unexposed people provides information about which cellular processes are involved in leukemia induction caused by benzene. Previously, we used a method called microarray to measure RNA levels of 20,000 specific genes simultaneously in the blood of 125 exposed and unexposed factory workers in China. We found a group of genes that were expressed differently in people exposed to benzene. Here, we used an innovative method called nCounter, which digitally counts the number of copies of RNA from up to 30 different genes simultaneously, to confirm these gene expression findings in the same workers. In a subset of samples, we used RNA sequencing, which measures the expression of all RNAs in a sample in a non-targeted way. This allowed us to search for non-human RNAs that could indicate infection. Among the RNAs that did not match known human genes, we detected bacterial and viral RNAs in two samples and we are examining if benzene affects which species are present. Ultimately, we want to use blood gene expression to further understand the underlying mechanisms of leukemia induced by benzene and to improve risk assessment of benzene in people exposed to a wide range of exposures, including environmental levels.

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TECHNICAL ABSTRACT

Folic Acid and Creatine as Therapeutic Approaches to Lower Blood Arsenic: A Randomized-Controlled Trial

Background: Over 140 million people worldwide are exposed to arsenic (As) through drinking water. Hepatic methylation of As facilitates urinary elimination. We have shown that 400ug folic acid (FA) daily improves As methylation and reduces blood As (bAs) in folate-deficient Bangladeshi adults. Urinary creatinine is also strongly associated with As methylation patterns. The biosynthesis of creatine (Cr), the precursor of creatinine, consumes half of all methyl donors and is downregulated by dietary Cr consumption.
Objectives: To determine whether supplementation with 400 or 800ug FA and/or Cr lowers bAs in a mixed folate sufficient/deficient population.
Methods: We conducted a 24-week RCT among adults in Bangladesh chronically exposed to As. Participants were randomized to placebo (N=102), 400ug FA (N=153), 800ug FA (N=151), 3g Cr (N=101), or 3g Cr+400ug FA (N=103) daily. At 12 weeks, half of the participants in each FA group were switched to placebo. All participants received an As-removal water filter at baseline.
Results: Linear models with repeated measures indicated that the decline in ln(bAs) in the 800µg FA group was greater than that of the placebo group over both 12 week periods (12 wks: b= -0.09, p=0.03; 24 wks: FA continued: b = -0.12, p=0.04; and FA switched to placebo: b = -0.14, p=0.02). There was no rebound in bAs due to cessation of FA supplements. Other treatments did not lower bAs significantly more than placebo.
Discussion: 800ug FA daily lowers bAs more than placebo in a mixed folate sufficient/deficient Bangladeshi population with a history of chronic As exposure.

LAY ABSTRACT

Nutritional supplementation to lower blood arsenic

Over 140 million people worldwide are exposed to arsenic through drinking water. After ingestion, arsenic is metabolized in the liver through a pathway (called “one-carbon metabolism”) that is dependent upon folate, a B vitamin. This metabolism is important for the elimination of arsenic from the body and involves the addition of methyl groups. The synthesis of creatine consumes half of all methyl groups available through the one-carbon metabolism pathway. We have shown previously that supplementation with 400 micrograms of folic acid daily improves arsenic metabolism and lowers blood arsenic concentrations in Bangladeshi adults who are deficient in folate. The objective of this study was to determine whether supplementation with 400 or 800 micrograms folic acid and/or creatine lowers blood arsenic in a population including both folate deficient and sufficient individuals. We conducted a 24-week study in which adults in Bangladesh who were chronically exposed to As were randomized to placebo, 400 micrograms folic acid, 800 micrograms folic acid, 3 grams creatine, or 3 grams creatine + 400 micrograms folic acid daily. At 12 weeks, half of the participants in each FA group were switched to placebo. All participants received a water filter at baseline that removes arsenic. Our results showed that the decline in blood arsenic in the group that received 800 micrograms folic acid was greater than that of the placebo group over the 24 week study. There was no rebound in blood arsenic due to stopping folic acid supplementation. The other treatments did not lower blood arsenic significantly more than placebo.

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TECHNICAL ABSTRACT

Biomarkers of organophosphorus exposures in humans

Organophosphorus (OP) compounds are widely used as insecticides and are responsible for poisoning among humans, especially agricultural workers. They inhibit cholinesterases and other serine hydrolases by binding to their active-site serines, leading to neurological damage and other serious health effects. Current methods for monitoring human OP exposures rely on enzymatic analyses and have several drawbacks. The partnership established with the Centers for Disease Control and Prevention (CDC) through the Superfund Research Program at the University of Washington has allowed us to develop mass spectrometric assays for biomonitoring OP exposures that are easily translated into the high-throughput protocols that the CDC currently uses for monitoring OP exposures. We have also contributed reference biomarker proteins and protocols to the CDC that will be useful for developing new methods. This collaboration has resulted in the isolation and characterization of plasma butyrylcholinesterase (BChE) and red blood cell (RBC) acylpeptide hydrolase (APH), and their cloning and recombinant expression in E.coli. The methodology developed consisted of immunomagnetic rapid isolation of the target biomarker followed by high-resolution mass spectrometry (LC-MS/MS) to identify methyl or ethyl OP-adducted active-site serine peptides from plasma, RBCs or dried blood spots (DBS). When tested with agricultural workers mainly exposed to chlorpyrifos, these methods detect as low as 2.5% of mono-ethyl phosphoserine modification of BChE. The use of DBS offers many advantages for collecting, shipping and storing samples. We have also purified RBC acetylcholinesterase and monocyte carboxylesterase.
Supported by NIH (P42ES04696, R01ES009883, ES09601/EPA-R826886, P41GM103533, T32ES007032); PNASH Center (U50OH07544-10); and the CDC.

LAY ABSTRACT

Detection of insecticide exposures in humans

Insecticides are responsible for poisoning among humans, especially agricultural workers. They inhibit the function of certain important proteins by attaching part of the structure of the insecticide to the protein. As a result, that protein loses its activity, leading to detrimental health effects. Current methods for monitoring human exposures to insecticides are not very accurate and present several drawbacks. A partnership established with the Centers for Disease Control and Prevention (CDC) through the Superfund Research Program at the University of Washington has allowed the development of improved assays for biomonitoring insecticide exposures that are easily translated into high-throughput protocols that the CDC currently uses for other related exposures. We have also provided reference materials to the CDC that are useful for their developing new methods. This collaboration has resulted in the isolation and characterization of two proteins from agricultural workers [butyrylcholinesterase (BChE) and acylpeptide hydrolase (APH)], that become modified by insecticide exposure. The methodology developed consists of rapid separation of the target protein from blood (drawn or collected on filter paper) followed by mass spectrometry (MS). MS is a technique that easily identifies modifications to proteins. We found that BChE and APH from agricultural workers had part of the insecticide chlorpyrifos attached to their structure. These methods have a much higher sensitivity than the currently used assays. The use of filter paper for blood sampling provides a significant advantage for collecting, shipping and storing samples. We are currently developing methods for monitoring two other proteins (acetylcholinesterase and monocyte carboxylesterase).

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TECHNICAL ABSTRACT

Transfer of triclocarban from mother to offspring through gestation and lactation

A variety of chemicals are not removed after wastewater treatment, which results in their release back into the environment and water supply. These may act as endocrine disrupting compounds (EDC), which may affect the function of the endocrine system and adversely affect offspring. Studies evaluating the effects of EDC during periods of development are lacking, including quantitative measures of accumulation after exposure. The focus of this work is to quantify the transfer and accumulation of the EDC, triclocarban (TCC), from mother to offspring after exposure in utero and through lactation using accelerator mass spectrometry (AMS). The high sensitivity of AMS allows for quantification of environmentally relevant concentrations of TCC (pM-nM).
We administered 14C-TCC to pregnant female mice through their drinking water (100nM). TCC was detected in offspring for both groups with the lactation group having a higher concentration (PND10 = 0.015%ID/g, ID = ingested dose) than the gestation group (GD18 = ~0.005%ID/g). Increases in offspring weight were also noted when compared to their control counterparts.
These data indicate that TCC is transferred from mother to offspring across the placental barrier and through lactation and that this exposure is associated with increases in offspring weight. While these effects were observed in mice, these findings suggest that if TCC is similarly transferred in humans, there may be implications after exposure on human health, in particular obesity. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABS-644431

LAY ABSTRACT

Transfer of triclocarban from mother to offspring through gestation and lactation

Many chemicals make it back into the water supply and the environment, even after wastewater treatment. Some of these chemicals may adversely affect endocrine function and offspring development; these chemicals are referred to as endocrine disrupting compounds (EDC). Studies evaluating the effects of EDC exposure during periods of development are lacking, therefore, in this study we are investigating if the EDC, triclocarban (TCC) can be transferred from mother to offspring during periods of development. In this work we are quantifying an environmentally relevant concentration (100nM) using a highly sensitive detection method, accelerator mass spectrometry (AMS).

TCC was administered to pregnant female mice through their drinking water during gestation and lactation. We found that TCC was capable of transferring from mother to offspring during both periods of exposure. We also observed an increase in offspring weight for both exposure groups. Although these effects were observed in mice, the data suggest that if TCC is similarly transferred in humans, there may be implications after exposure on human health, in particular obesity. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABS-644431

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TECHNICAL ABSTRACT

Application of 96 Well Plate Solid Phase Extraction to Increase the Throughput of Regulatory Lipid Mediators Metabolomic Profiling in Plasma

Our previous studies showed that the environment pollutes such as PCBs and dioxins alter the regulatory lipid mediators. These regulatory lipid mediators play important roles in various biological functions such as angiogenesis, proliferation, apoptosis and etc. A comprehensive profiling method for monitoring these mediators is very helpful to help us understand how environment pollutes affect human health. We have a scheduled MRM LC-ESI MS/MS method to profile these lipid mediators. In 21.5 minutes, 87 regulatory lipid mediators were measured simultaneously. However, the sample preparation method-SPE method is labor extensive and time consuming. Here, we report a method for profile the regulatory lipid mediators using 96 well plate format SPE and Phree plate to provide the higher throughput than the original method. The regulatory lipid mediators' standards spiked into human plasma at various concentrations (3 nM, 30 nM and 300 nM). The samples were extracted through 96 well plate format of Oasis HLB uElute 96 well plates or Phree Phospholipid Removal 96-well plate. The extraction eluents were mixed with loading internal standards in a 96-well storage plate before loading on the LC/MS/MS for the separation and detection. There is significant improvement for the throughput compared to original method (4 plates x 96 than 40 SPE cartridge per day) without significant loss of recovery rate for the extraction. The reproducibility of the new methods are 80-120%.

LAY ABSTRACT

Our previous studies showed that the environment pollutes such as PCBs and dioxins alter the regulatory lipid mediators. These mediators work like the hormone in the human beings. A good method to monitor these mediators is very helpful to understand how pollutes affect human health. In our past city, we developed a sensitive and accurate method for this. However, the speed of the analysis becomes a hurdle when the big sample set need to be done. Here, we reported a method to speed up the whole process. In the new method, the extraction work is done in parallel by using 96-well plate format. There is significant improvement for the throughput compared to original method (4 x 96 versus 40 samples per day) without significant loss of recovery rate for the extraction. The reproducibility of the new methods is 80-120%.

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TECHNICAL ABSTRACT

The commonly used antimicrobial additive triclosan is a liver tumor promoter

Triclosan (TCS) is a synthetic, broad-spectrum antibacterial chemical used in a wide range of consumer products including soaps, cosmetics, therapeutics, and plastics. The general population is exposed to TCS due to its prevalence in a variety of daily care products as well as through water-borne contamination. TCS is linked to a multitude of health and environmental effects ranging from endocrine disruption and impaired muscle contraction to impacts on aquatic ecosystems. We discovered that TCS was capable of stimulating liver cell proliferation and fibrotic responses, accompanied by signs of oxidative stress. Through a reporter screening assay with an array of nuclear xenobiotic receptors (XenoRs), we found that TCS activates the nuclear receptor constitutive androstane receptor (CAR) and, contrary to previous reports, has no significant effect on mouse PPARα. Using the procarcinogen diethylnitrosamine (DEN) to initiate tumorigenesis in mice, we discovered that TCS substantially accelerates hepatocellular carcinoma (HCC) development, acting as a liver tumor promoter. TCS-treated mice exhibited a large increase in tumor multiplicity, size, and incidence compared to control mice. TCS-mediated liver regeneration and fibrosis preceded HCC development and may constitute the primary tumor promoting mechanism though which TCS acts. These findings strongly suggest that there are adverse health effects in mice with long-term TCS exposure, especially on enhancing liver fibrogenesis and tumorigenesis, and the relevance of TCS liver toxicity to humans should be evaluated

LAY ABSTRACT

Triclosan promotes liver tumor growth in mice

Triclosan (TCS) is a broad spectrum antimicrobial agent and has become one of the most common additives used in consumer products including many household and personal care products. As a result, TCS has significantly impacted the environment and has been frequently detected in human body fluids.Through a long-term feeding study, we found that TCS enhances hepatocyte proliferation, fibrogenesis, and oxidative stress, which, we believe, can be the driving force for developing advanced liver disease in mice. Indeed, TCS strongly enhances hepatocarcinogenesis following treatment of a tumor initiator, accelerating malignant liver tumor development. Consequently, TCS-treated mice exhibited increased liver damage and histological alterations, suggesting that TCS is responsible for liver injury that leads to disrupted liver integrity and compromised function. While animal studies require higher chemical concentrations than predicted for human exposure, this study demonstrates that TCS acts as a LIVER tumor promoter, and the mechanism of TCS-induced mouse liver pathology may be relevant to humans.

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TECHNICAL ABSTRACT

Endocrine disrupting chemicals in dust: a pilot study from Los Laureles Canyon, Mexico.

Indoor dust is an important pathway of environmental exposure of endocrine disrupting chemicals (EDCs). In fulfillment of UCSD Superfund Community Engagement Core's aim of identifying, prioritizing and addressing environmental health hazards in the San Diego-Tijuana city-region, we conducted a study to measure phthalates (PAEs), triclosan (TCS), bisphenol A (BPA), and trace metals from indoor dust collected from two neighborhoods in Los Laureles Canyon, Mexico. We surveyed and collected dust from 46 houses using dust collector filters attached to cordless vacuums. Heavy metals, TCS, PAEs, and BPA were detected in all samples. The geometric mean (GM) and 95% confidence intervals for selected dust heavy metal concentrations were as follows: arsenic 4.9 µg/g (4.3 – 5.6); cadmium 0.6 µg/g (0.41-0.73); and lead 25.1 µg/g (17.7-35.4). GMs and 95% confidence intervals for dust concentrations of organic compounds, including 6 common phthalate metabolites were as follows: TCS 92.7 ng/g (53.5 – 160.5); BPA 580.4 ng/g (364.1 – 925.2); dimethyl phthalate 86.2 ng/g (61.4 – 120.9); diethyl phthalate 728.4 ng/g (518.3 – 1,023.7; dibutyl phthalate 5,797.6 ng/g (4,167.8 – 8,064.6); benzylbutyl phthalate 1,743.1 ng/g (1,198.3 – 2,535.7); bis-2 ethylhexyl phthalates 108,430.9 ng/g (81,950 – 143,468.8); and di-n-octyl phthalates 841.0 ng/g (655.1 - 1,079.8). Although this is a preliminary proof of concept study, the first of its kind in an urban area of Mexico, it demonstrates wide variability in levels of EDCs in dust. TCS, BPA and PAEs concentrations in household dust were lower than that of other studies. This poster will highlight the methodology and include additional analysis and statistical comparisons.

LAY ABSTRACT

Evaluation of harmful chemicals found in dust collected from homes in Los Laureles Canyon, Tijuana Mexico.

Indoor dust can contain chemicals that affect the endocrine system, known as endocrine disrupting compounds. These chemicals include: bisphenol A and phthalates, commonly found in household products including plastics; triclosan, an antibacterial agent commonly found in personal care products, and; heavy metals, commonly found in industrial wastes, vehicle emissions, paints and treated woods. In fulfillment of UCSD Superfund Community Engagement Core's aim of identifying, prioritizing and addressing environmental health hazards and issues in the San Diego-Tijuana city-region, we conducted a small study to collect indoor dust and measure concentration levels of phthalates, triclosan; bisphenol A, and heavy metals from two neighborhoods in Los Laureles Canyon, San Bernardo and Cardenas, as a preliminary study for a much larger environmental epidemiological study. We surveyed residents and collected dust from 46 houses using a Dustream® dust collector filter attached to the end of a cordless vacuum. Heavy metals, triclosan, bisphenol A, and phthalates were detected in all samples. Average dust concentration levels, including the range of concentration levels of each endocrine disrupting compound and heavy metal will be presented. This is the first study of this kind conducted in Mexico. This poster will highlight the study methodology, dust analysis, and statistical comparisons of the chemicals.

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TECHNICAL ABSTRACT

Using GIS Technology to Inform Environmental Health Policy: A Case Study Involving USEPA's Decision-Making Process of Remedy Selection and Building Community Capacity

Purpose To assess the adequacy of USEPA remedy selection, and build a community's capacity to use GIS to participate effectively in this decision-making process.
Background. The use of geographic information systems (GIS) technology is not widely used in site assessment. USEPA must engage the public as stakeholders throughout this science-driven process. As non-scientists, community organizations operate at a disadvantage. Collaboration with academia provides communities with access to technical resources and content expertise, thus building their capacity to participate effectively.
Methods. A community-based participatory process identified community concerns about USEPA's remedy selection. Researchers conducted document analysis, analyzed data on flooding frequency and magnitude, and used geospatial data to generate microtopographical figures of surface water flow and flood simulation. Direct visual site observations documented human activity. Community members evaluated their ability to engage effectively with regulators.
Results. Microtopography analysis revealed a complex drainage network within the river oxbow downstream of the Superfund site. Ever-increasing flood magnitudes have impacted this river. Computer simulation demonstrated the extent of flooding that would occur. Researchers found evidence of undocumented human activity and a dearth of environmental sampling in these areas.
Conclusions & Implications. GIS provided visual clarity and scientific substantiation and played a transformative role in public participation. Enhancing community capacity facilitates a more equitable voice in defining issues and promoting effective and long-term solutions for hazardous waste sites.
Acknowledgements. NIH/NIEHS P42-ES013660; USGS G10PC00026; LiDAR G10PD01027/ARRA, G10PD02143/non-ARRA. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIH/NIEHS.

LAY ABSTRACT

Using GIS Technology Helped A Community Organization Argue with the Government

Why We Did This. To assess what the United States Environmental Protection Agency (USEPA) wants to do to clean up a polluted site and to help a community present their case successfully to USEPA. Not many people use GIS technology to assess really polluted sites. USEPA is legally required to involve community members in the process of deciding how to clean up the river. Because this process involves a lot of science, the community is at a real disadvantage. One community asked a local university for help so they could present their views and concerns to the government.
How We Did This. The community told us their concerns about the government's cleanup plan. We searched documents to identify and analyze area flooding, and used geospatial information to create incredible pictures of surface water flow and floods. We took pictures to show that people are going where they're not supposed to go.
What We Found Out. We found a network of surface water downstream from the polluted site. Floods have happened more frequently over the last decade
and they are getting worse. Computer graphics showed how much this area would flood into neighborhoods. USEPA didn't take soil and water samples where humans have been going.
So What? Using this technology helps communities to argue their case with the government really well.
Where We Got Our Money. NIH/NIEHS P42-ES013660; USGS G10PC00026; LiDAR G10PD01027/ARRA, G10PD02143/non-ARRA. Don't blame NIH/NIEHS.

72	Lei Wu, Post-doctoral scholar, Department of Earth and Environmental Science, University of Pennsylvania, leiwu1@sas.upenn.edu Environmental Sciences and Engineering Lei Wu and Douglas J. Jerolmack Department of Earth and Environmental Science, University of Pennsylvania, PA 19104

TECHNICAL ABSTRACT

Pore-scale mechanisms of asbestos fiber deposition and mobilization during steady and transient flow through saturated porous media

The unusually large aspect ratio of asbestos fibers is expected to exert a strong control on the migration and trapping of particles in groundwater transport through soil; no studies, however, have examined aqueous transport in the laboratory. In this work, we propose pore-scale observation of asbestos fiber mobilization, transport, and retention within a transparent flow cell packed with saturated quartz sand of various sizes (710, 360, 240 and 150 um) and at several flow rates (from 0.0625 cm/min to 0.625 cm/min). Basic physico-chemical processes (e.g., straining and immobile capture) controlling asbestos fiber deposition and mobilization will be elucidated. Key factors (e.g., solution chemistry and flow regime) that have effects on the basic processes will be identified. Theoretical models will be tested and refined to predict our laboratory observations of transport and straining of asbestos fibers in saturated porous media. These pore-scale results will shed light on the effect produced by large aspect ratio and its role in asbestos fiber mobilization and retention within porous media. In addition, these results may help us understand how to incorporate straining process into transport modeling of fiber-like particles.

LAY ABSTRACT

Finding how asbestos fibers travel in groundwater

We know that asbestos fibers can travel in groundwater and rivers, which is a significant pathway for the spread of asbestos. Such movement has threatened drinking water supplies in some areas. We have limited information, however, about how asbestos fibers travel in groundwater and rivers. To determine this, we designed a realistic micro-sized flow cell of porous media that allows direct observation of asbestos fiber movement and straining at pore scale. By using this experimental set-up, we are able to elucidate basic physico-chemical processes (e.g., straining and immobile capture) controlling asbestos fiber deposition and mobilization, and identify key factors (e.g., solution chemistry and flow regime) that have effects on the basic processes. Based on our results, we also plan to develop theoretical models to predict the movement of asbestos fibers in groundwater. This work will help us to identify the extent to which asbestos fibers at the Ambler Superfund site can move within groundwater, and let us make recommendations for the containment of asbestos in order to limit aqueous transport.

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TECHNICAL ABSTRACT

Exploring mycorrhizal fungi and plants as agents in altering asbestos fibers and in remediating asbestos-contaminated soils

Current state-of-the-art for treatment of asbestos-contaminated sites is to move the asbestos and/or cap the site. To remediate asbestos piles in situ, one must seal the asbestos to decrease its transport. Generally, the particular length to width ratio of asbestos fibers cause [1] inflammation in the lung and GI tract and make the fibers difficult to expel, and [2] reactive iron in the fibers contribute oxidative damage to DNA, which can lead to mesothelioma.
Asbestos is a colloquial term encompassing several fibrous silicates classed in two mineral families: amphibole and serpentine. In most contaminated sites (chrysotile form), iron (generally as Fe2+) substitutes for Mg in the octahedral layer, and the toxicity likely scales with iron abundance. This project takes a novel approach to the remediation of asbestos-polluted soil by using biological organisms.
We will test serpentine ecotypes of several native warm season grasses known to be highly mycotrophic (require AMF) and other plant species that accumulate heavy metals but do not all form mycorrhizae. We will also employ several AMF species, including some collected from non-serpentine soils. The experiment will vary AMF community, five host plant species and type of asbestos. We will evaluate the efficacy of plants and their associated AMF in harvesting Fe atoms from asbestos particles and changing the structure of the fibers, modifications rendering them less toxic.
We will better understand the total effect of AMF and metal hyper-accumulator plants on iron mobility in soils containing asbestos.

LAY ABSTRACT

Exploring mycorrhizal fungi and plants as agents in altering asbestos fibers and in remediating asbestos-contaminated soils

The proposed research specifically addresses whether and (1) how fast free asbestos fibers in the environment or under EPA-installed caps chemically and structurally change over time and (2) how this fiber weathering can be accelerated to remediate asbestos-contaminated sites in a timely and cost-effective manner. The proposed research examines whether chemical alteration of asbestos particles by plants and/or fungi, either directly or indirectly via plant exudates or fungal exudates, may be useful for bioremediation of asbestos-contaminated sites.
The roots of most plant species associate with mycorrhizal fungi that help facilitate nutrient uptake and in other capacities. The fungi, in turn, rely on the plant for their source of carbon and cannot survive without a plant host. Arbuscular mycorrhizal fungi (AMF) occur on most plants; severalspecies known to accumulate heavy metals are exceptions. Indeed, the project targets plant species that are either metal accumulators, known to take up metals in large quantities, or are native to soils naturally high in heavy metals (serpentine) and whose roots form mutually beneficial relationships with mycorrhizal fungi. We hypothesize that a combination of iron accumulator plants and plants that form symbioses with AMF, which may not be the same, will alter the chemical composition of asbestos fibers.
By measuring iron concentrations, we will determine which species of AMF or plant accumulator (or combination) best removes iron from asbestos particles. The reduced toxicity of the chemically altered asbestos fiber will be validated, in part by cooperation with other biomedical SRP projects.

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TECHNICAL ABSTRACT

Development of a Sensitive Immunoassay for the Herbicide Paraquat Using a Heavy-Chain Single Domain Antibody from Alpacas

Paraquat, a widely used herbicide, is a fast-acting and non-selective contact chemical that works by inhibiting photosynthesis in plants. Paraquat is known to be highly toxic to humans upon oral ingestion and has caused more deaths than any other agricultural product. Previously, a sensitive polyclonal based immunoassay for the detection of paraquat in human samples was developed, however polyclonal antibodies have many limitations. The single domain antibodies from camelids represent a new technology in small molecule immunoassays with improved assay sensitivity, improved stability, field portability and high-throughput nature. An alpaca was immunized with paraquat hapten and single domain antibodies specific to paraquat were screened through phage display and amplified and purified. A homologous indirect competitive ELISA with an antibody clone produced 9.2 ng/mL assay sensitivity. To obtain a more sensitive and stable nanobody, we are trying to select with heterogeneous coating antigen. An excellent nanobody could be used in environmental monitoring, human exposure determination, as well as to toxicity prevention.

LAY ABSTRACT

Simple, Rapid, Sensitive Immunoassay for Herbicide Paraquat Detection

Paraquat, which is a charged, water-soluble compound, has resulted in more pesticide related deaths worldwide primarily via intentional ingestion. Recently reregistered in the U. S. as a new and safer formulation, its use is anticipated to increase dramatically. Therefore, the need for an efficient detection method to monitor its overuse and human exposure is urgent. Immunoassay technology, which has a low limit of detection and thus permits quantification at trace levels, is a simple tool for detection and quantification of low-molecular-weight contaminants, allowing relatively fast, high-throughput analysis. So far, all of the previously reported immunoassays for paraquat are based on conventional polyclonal antibodies and monoclonal antibodies. However, preparation of high quality antibodies is still a bottleneck problem and appears to be increasingly important when establishing immunoassay methods. Advances in recombinant DNA technology have provided an alternative approach by allowing the engineering of recombinant antibodies with desirable affinity and specificity. The single domain antibodies from camelids with improved assay sensitivity, improved stability, field portability and high-throughput nature are the most potential reagent to develop simple, rapid, sensitive detection methods for small chemical contaminant. We are trying our best to get the desired recombinant camelids antibody and establish efficient detection methods for paraquat in this work.

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Environmental Sciences and Engineering

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TECHNICAL ABSTRACT

PCB-mediated estrogen receptor-α-dependent histone modifications: Possible regulatory link between PCB exposure and induction of vascular inflammation

Vascular endothelial cell dysfunction and chronic inflammation are critical events in the pathology of atherosclerosis throughout life. Exposure to persistent environmental pollutants, such as polychlorinated biphenyls (PCBs), may cause inflammation of the vascular endothelium leading to the development of atherosclerosis. In this study, we found that coplanar PCBs 77 and 126 induced the nuclear factor-κB (NF-κB) subunit p65 and its target genes such as interleukin (IL)-6 and IL-1β, monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and C-reactive protein (CRP), which all have been implicated in promoting atherosclerosis. Interestingly, PCBs 77 and 126 also upregulated the expression of jumonji domain-containing protein 2B (JMJD2B), a histone H3K9 trimethyl demethylase, in an estrogen receptor (ER-α)-dependent manner and further caused corresponding histone modifications. Thus, we propose that coplanar PCBs are atherogenic through NF-κB pathway mediated inflammation, which is regulated by ER-α-dependent histone modifications. Further studies will focus on ER-α and JMJD2B knockdown, impacts of PCBs on NF-κB pathway, as well as chromatin immunoprecipitation (ChIP) to access p65 binding on promoter regions of target genes. This work may have implications in understanding epigenetic regulation of PCB-induced vascular toxicity in the individual and perhaps even transgenerationally. (NIH/NIEHS P42ES007380)

LAY ABSTRACT

PCBs may cause vascular disease and inflammation through epigenetic mechanisms

In human vascular diseases, dysfunction of the inner lining of blood vessels (endothelium) is thought to be a key event in the development of heart disease. Endothelial dysfunction is associated with increased levels of pro-inflammatory mediators. This dysfunction and inflammation could result from exposure to environmental factors, such as persistent pollutants, e.g., polychlorinated biphenyls (PCBs). There are multiple pathways that can lead to inflammatory events, however, we do not know exactly how PCB-mediated inflammation of the vascular system is biologically regulated. In this study, we found that PCBs upregulated the expression of a master regulator of inflammation, nuclear factor-κB (NF-κB) subunit p65 and some of its target genes. Increased levels of these genes all are risk factors in the development of heart disease. Interestingly, PCBs may also cause toxicity through novel regulatory epigenetic mechanisms. Epigenetics is a new field of study that may help to explain heritable and non-heritable health outcomes. We determined that PCBs can alter multiple epigenetic modification patterns, which could help to explain regulatory mechanisms of PCB-induced inflammation and heart disease in human populations. (NIH/NIEHS P42ES007380)

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TECHNICAL ABSTRACT

LAY ABSTRACT

	Methylmercury bioaccumulation in an urban estuary Methylmercury (MeHg) concentrations in sediment, water, invertebrates and fish were measured at 10 sites within the Delaware River Estuary. Sample sites ranged from Philadelphia, PA to Cape May, NJ and were chosen to span spatial gradients of contamination, salinity and human use, factors that may influence MeHg availability and trophic transfer. MeHg bioaccumulation data are presented for blue crabs, grass shrimp, killifish, and white perch and relationships between biotic MeHg concentrations and varying salinity and urban development are explored.

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TECHNICAL ABSTRACT

Development of an enzyme-linked immunosorbent assay (ELISA) for tetramethylenedisulfotetramine (TETS)

LAY ABSTRACT

Development of an immunoassay for detection of a rodenticide

Tetramethylenedisulfotetramine (TETS) is one of the most toxic substances known. It had been used as a rodenticide until it was banned in 1984 because of its high toxicity to humans. Because it is easy and cheap to prepare it is still available illegally. In the past fifteen years there have been a few hundred people poisoned by TETS worldwide. Due to its remarkable stability under normal environmental conditions, sites contaminated with TETS present a hazard to animals and humans for a long period of time. Present methods for detection of this rodenticide are laborious and time consuming. Thus development of new, cheap and easy methods for detection of TETS is highly desirable. The enzyme-linked immunosorbent assay (ELISA) perfectly fits these requirements. ELISA is a test that uses antibodies and color change to identify a substance. In the present report we show our progress on the development of an ELISA for the detection of TETS.

78	Yongquan Lai, Post-doctoral scholar, Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, yqlai@live.unc.edu Environmental Sciences and Engineering Yongquan Lai, Wanda Bodnar, Hadley Hartwell, James A Swenberg* Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, North Carolina, United States

TECHNICAL ABSTRACT

Identification and Differentiation of Endogenous and Exogenous DNA-Protein Crosslinks in Nonhuman Primates and Rats Following Inhalation Exposure to Stable Isotope Labeled Formaldehyde

Formaldehyde (FA) causes squamous cell carcinomas in the nasal passages of rats and has been classified as a known human carcinogen by IARC. While the formation of FA-induced DNA-protein crosslinks (DPCs) has long been thought to be a major form of DNA damage, it has not been possible to differentiate between exogenous and endogenous DPCs. Here, we identify the first specific FA-induced DPCs with the chemical structure of a methyl linkage between dG and cysteine (dG-Me-Cys) in vivo. A highly sensitive nano-LC-MS/MS method was developed to determine the distribution of endogenous and exogenous DPCs in nonhuman primates (NHPs) and rats following inhalation exposure to 6 ppm and 15 ppm of [13CD2]-FA, respectively. The results indicate exogenous DPCs were only found in respiratory sites of both NHPs and rats, while endogenous DPCs were presented in all tissues tested, including critical tissues such as PBMC and bone marrow. Specifically, exogenous and endogenous DPCs were detected at 1.36 and 3.76/10^8 dG in NHPs noses, respectively. Accumulation of exogenous DPCs was observed in rat noses over the 4 days exposure. The lack of exogenous DPC formation at distant sites further demonstrates that inhaled FA does not cause systemic genotoxic effects, thereby strengthening implausibility that inhaled FA increases the risk of leukemia. In contrast, the presence of relatively high amounts of endogenous DPCs in all examined tissues offers an important perspective on the potential health risks posed by endogenous FA. This study provides strong additional evidence for science-based risk assessment of inhalation exposure to FA.

LAY ABSTRACT

Differentiating Endogenous and Exogenous DNA-Protein Crosslinks in Nonhuman Primates and Rats Following Inhalation Exposure to Formaldehyde

Formaldehyde causes squamous cell carcinomas in the nasal passages of rats and has been classified as a known human carcinogen. Recent epidemiological data has also suggested a possible link between formaldehyde exposure and increased risk for leukemia. However, whether or not formaldehyde causes leukemia remains debatable. There are inconsistent epidemiology studies and divergent assumptions regarding the systemic bioavailability of inhaled formaldehyde. The issue of whether inhaled formaldehyde can reach to the systemic circulation is an important factor for assessing risk of adverse outcome at nonrespiratory sites. In addition, the natural endogenous presence of formaldehyde in cells complicates assessment of any risk posed by inhaled formaldehyde. While the formation of formaldehyde-induced DNA-protein crosslinks (DPCs) have long been thought to be a major form of DNA damage, it has not been possible to differentiate between exogenous and endogenous DPCs. Here, we present the first report that not only identifies the specific chemical structure of formaldehyde-induced DPCs in vivo, but also differentially quantified the endogenous and exogenous DPCs in nonhuman primates and rats following inhalation of known amounts of formaldehyde. The lack of exogenous DPC formation at distant sites demonstrates that inhaled formaldehyde does not cause systemic genotoxic effects, thereby strengthening implausibility that inhaled formaldehyde increases the risk of leukemia. In contrast, the presence of relatively high amounts of endogenous DPCs in all examined tissues offers an important perspective on the potential health risks posed by endogenous formaldehyde. This study provides strong additional evidence for science-based risk assessment of inhalation exposure to formaldehyde.

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TECHNICAL ABSTRACT

Nanobody-based immunosensors for the sensitive and selective detection of BDE-47

Rapid, sensitive, and cost-effective chemical detection can greatly assist in determining exposure to hazardous substances. Two immunosensors for the detection of a chemical flame retardant using single domain antibodies (sdAb, nanobodies or VHH) isolated from an alpaca have been developed. SdAb can be produced rapidly and cheaply in bacterial cultures, while also maintaining excellent selectivity for a desired chemical target. One specific chemical flame retardant congener, 2,2',4,4'-tetrabro-minated diphenyl ether (BDE-47), is often the major poly-BDE (PBDE) congener present in human and environmental samples and that which is the most frequently detected. The assay sensitivity of the developed immunosensors based on either electrochemical impedance spectroscopy (EIS) or smartphone-interfaced microfluidic lab-on-a-chip (LOC) formats was down to the part-per-billion (microgram per liter) level for detecting BDE-47. The EIS format allows for detection using a label-free format, thus reducing the number of reagents needed. The LOC format is interfaced with a smartphone to allow non-technical users access and ease of sampling. The use of this sdAb reagent in these two sensor platforms for biosensing demonstrates the versatility of VHH antibodies.

LAY ABSTRACT

Developing label-free electrochemical impedance and smartphone-interfaced microfluidic immunosensors for the detection of a chemical flame retardant using single domain antibodies

Polybrominated diphenyl ethers (PBDEs) are a class of compounds used as flame retardant additives. They widely exist in electronics, furniture foam, and plastics. Due to the greater potential to leach from the original product during their lifetime, the health hazards of PBDEs have attracted increasing scrutiny, even with several formulations banned recently. However, the human and environmental monitoring programs are often limited by the cost and complexity of sample testing. It is of great significance to develop rapid, sensitive, and cost-effective detection methods for PBDEs. Utilizing the smart single domain antibodies (sdAb, nanobodies or VHH) isolated from an alpaca, we developed two immunosensors to trace the 2,2',4,4'-tetrabro-minated diphenyl ether (BDE-47), often the major and the most frequently detected PBDEs congener present in human and environmental samples. One immunosensor is based on the electrochemical impedance spectroscopy (EIS), which varies according to the varying concentration of the target analyte present due to the change of impedance when the analyte is captured by the BDE-47 antibodies on the electrode. The other is a smartphone-interfaced microfluidic lab-on-a-chip (LOC) format, which transfers the classical bench ELISA method to the small chip, allowing non-technical users to easily perform the sampling and detection through the smartphone. The assay sensitivity of the both developed immunosensors was down to the part-per-billion (microgram per liter) level for detecting BDE-47, demonstrating the versatility of VHH antibodies in these two sensor platforms for biosensing.

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TECHNICAL ABSTRACT

Hapten synthesis and antibody development for herbicide 2,4-D immunoassay

2,4-Dichlorophenoxyacetic acid (2,4-D) is a phenoxy herbicide used in the control of broadleaf weeds. It is one of the most widely used herbicides in the world. Antibodies against 2,4-D have been produced. However, all of them use 2,4-D as the hapten, coupled to protein through the carboxyl group. In this research, 4 novel haptens were designed, synthesized and conjugated with protein as immunogen and coating antigens, and polyclonal antibodies were obtained from rabbits immunized with these conjugates. With the screen of homologous and heterologous coating antigens, the antibodies 1518# and 1520# showed high sensitivity for the 2,4-D. The IC50 was 2.66 and 0.97 ng/mL, respectively. There were no obvious cross-reactivities with most of the structural analogues. The recoveries of 2,4-D from tap water and urine samples ranged from 78-127%. These results indicate that the ELISA could be a convenient and supplemental analytical tool for monitoring 2,4-D residues in environmental and human samples.

LAY ABSTRACT

Immunoassay method for the detection of the herbicide 2,4-D

2,4-Dichlorophenoxyacetic acid (2,4-D) is a herbicide used to control broadleaf weeds. It is one of the most widely used herbicides in the world. The U.S. Department of Agriculture approved the commercial sale of genetically engineered corn and soybean seeds that can withstand application of the herbicides 2,4-D and Roundup. It is expected that the use of 2,4-D will greatly increase in the next few years. The widespread use of 2,4-D and associated health concerns have made monitoring of environmental samples for the presence of 2,4-D desirable. Although conventional i methods are accurate, they require expensive instruments and time-consuming sample treatments before analysis. In contrast, immunoassays, based on antibodies are rapid and cost effective and allow high sample throughput. In this study, we applied novel chemistry to obtain two antibodies that can detect very low levels of 2,4-D specifically. To evaluate the assay we added known amounts of 2,4-D to tap water or urine samples. When the samples were analyzed by immunoassays we successfully detected an average of 78–127% of the amount we added. Over all, the developed method is a quick, and effective method for monitoring for 2,4-D in the environment and people.

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TECHNICAL ABSTRACT

LAY ABSTRACT

Low levels of oxidative stress causes clustered DNA damage leading to DNA double strand breaks and mutations

One of the major public concerns about Superfund chemicals is their carcinogenicity. Oxidative stress has been implicated in carcinogenesis, aging and in the development of chronic and degenerative diseases such as arthritis, autoimmune disorders, cardiovascular & neuro-degenerative diseases. Although environmental heavy metals, PCBs, dioxin and polycyclic aromatic hydrocarbon metabolites cause oxidative DNA damage, little is known about the precise mechanism by which oxidative stress induces gene and chromosomal mutations. Most human tumors are characterized by large genome rearrangements. In addition, oxidative stress under antioxidant depletion causes DNA deletions in mice. These previous results suggest that reactive oxygen species (ROS) may cause gene and chromosome mutations through DNA double strand breaks (DSBs). However, the generation of DSBs by ROS is controversial. Here we have shown that H2O2 at physiologically-relevant levels causes a marked increase in clustered DNA lesions (closely spaced damages on DNA) leading to significant increases in DSBs. Furthermore, a reverse genetic approach revealed a significant contribution of the error prone DSB repair pathway in H2O2-induced DNA damage response and mutagenesis. Our results indicate that H2O2, even at low levels, can cause deleterious clustered DNA lesions that further lead to DSBs with complex DNA ends. Repairing such complex DSBs with the error prone DSB repair pathway increases the likelihood that mutations will result. This genomic instability induced by low levels of ROS can be involved in spontaneous mutagenesis and the etiology of a wide variety of human diseases caused by environmental reactive agents.

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TECHNICAL ABSTRACT

Development of an Immunoassay for the Detection of the Phenylpyrazole Insecticide Fipronil and its Metabolites

Phenylpyrazole insecticides such as fipronil have been used as replacements for organophosphates. Fipronil's wide application raises concern about environmental contamination and risk for fish, birds, other non-targeted beings and human health. Sensitive enzyme-linked immunoassays (ELISA) were developed using antibodies with different specificities to fipronil and its metabolites. Two ELISAs having IC50 values of 0.58 ± 0.06 and 2.6 ± 0.4 ng/mL were developed. Design of different haptens and coating antigens resulted in two assays with distinct cross-reactivity patterns for environmental and biometabolites: 99.4%, 51.6% and 110.2% vs 40.7%, 2.0% and 27.6 % for fipronil-sulfide, fipronil-detrifluoromethylsulfonyl and fipronil-desulfinyl, respectively. Immunoassay performance was demonstrated by a recovery study from a spiked human serum matrix, giving recovery values in the range of 93-111% for different concentrations. The assay has the sensitivity to measure fipronil and its analogs in serum at levels relevant for exposure monitoring.
However, urine metabolites are more attractive markers of exposure to chemicals. For the first time we have synthesized fipronil hydroxide, a recently identified metabolite in rat urine. It is being studied in insect assays for bioactivity and toxicity. The synthesized standard helps to elucidate the usefulness of fipronil hydroxide as a urinary marker of fipronil exposure in humans.
The fipronil immunoassay was transferred in a lateral flow format. Such rapid detection tools could be applied to monitoring of population exposure occurring in the home, thus preventing undesirable consequences of fipronil exposure. Routine environmental monitoring could also help in timely detection of environmental contamination preventing at-risk species from exposure.

LAY ABSTRACT

Development of a Rapid Test for the Detection of the Insecticide Fipronil and its Metabolites

Fipronil is a highly effective insecticide used by lawn care and pest control operators, to treat golf courses and food handling establishments, and in constructing buildings. Fipronil is registered for use on fruits, vegetables, coffee, tea, rice, other crops, and in seed treatments. It is widely used in topical pet care products. Fipronil's widespread uses raise concern about environmental contamination and risk for fish, birds, other non-targeted beings and human health. We have developed rapid, sensitive tests based on antibodies (enzyme-linked immunoassays, ELISA) that measure fipronil and products of its break down in the environment or the human body. The immunoassay can measure fipronil or its breakdown products in human serum at levels relevant for monitoring exposure in people.
However, urine is easier to collect from people than blood, so measuring fipronil or its breakdown products in urine is more desirable. For the first time we have synthesized fipronil hydroxide, a recently identified metabolite in rat urine. It is being studied on insects for bioactivity and toxicity. The synthesized standard helps to elucidate the usefulness of fipronil hydroxide as urine marker of fipronil exposure in humans.
The fipronil test has been transferred to a dipstick format similar to a pregnancy test. Such rapid detection tools could be applied to monitoring of population exposure occurring in the home, thus preventing undesirable consequences of fipronil exposure. Routine environmental monitoring could also help in timely detection of environmental contamination preventing at-risk species from exposure.

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TECHNICAL ABSTRACT

Establishing a three electrode system for the electrochemically-induced reduction of trichloroethylene

Electrochemical reduction is a fast and effective method to remove trichloroethylene (TCE) from contaminated groundwater. While iron anodes have been proven to improve the removal TCE in a mixed electrolytic system, they cause high pH and precipitation. In this study, we evaluated the use of a novel three electrode system; by using an additional inert anode (mixed metal oxide, MMO), to overcome drawbacks of using a system of single iron anode. The treatment was conducted under anode (MMO)→anode (iron)→cathode (iron) arrangement in a flow-through column. We tested 15/45 mA and 30/30 mA current split ratios between MMO and iron anode under 60 mA current intensity. Compared to the system without MMO anode, the 30/30 ratio decreased precipitation by 20% while the removal efficiency of TCE was doubled without a significant change in pH. By reducing the precipitate formation, less cathode surface is covered by the particles thus leaving it available for TCE reduction. Further, we found that increased current intensity to 90 mA and 120 mA, improved the TCE removal compared to 60 mA by 12% and 13%, and reduced precipitation formation by 30% and 42%, respectively. However, the higher currents cause an increase of pH to 11. The results of this study show that optimization of anode→anode→cathode arrangement overcome the drawbacks of the use of a single iron anode and increases the removal rate of TCE. This process will allow implementation of an efficient, solar-powered and practical electrochemical system for in situ treatment of contaminated groundwater.

LAY ABSTRACT

Using a direct electric current to clean groundwater

We manipulate groundwater chemistry in wells by applying low direct electric current through electrodes. By using the proper electrode materials and arrangements, we can create conditions in groundwater that remove contaminants such as chlorinated solvents (e.g., trichloroethylene, or TCE). However, it is important to maintain the natural groundwater conditions after the treatment. In this study, we evaluated the use of a novel three electrode system with two different anode materials: iron to create conditions to remove TCE, and mixed metal oxide as an inert material to maintain natural groundwater chemistry. The system was successful in TCE removal (77%) while groundwater conditions remained controlled after the treatment. This process will allow implementation of an efficient and practical electrochemical system for in situ, or in place, treatment of contaminated groundwater.

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TECHNICAL ABSTRACT

Radicals from the Gas-Phase Pyrolysis of Lignin Model Compounds. 1. p-Coumaryl and Cinnamyl Alcohols

Lignins are pyrolyzed to produce a mixture of phenols which retain structural features of the original polymer. A number of analytical-pyrolysis studies on lignocellulosic materials show that the cinnamyl alcohols are also primary products along with the main monolignols – p-coumaryl, coniferyl and sinapyl alcohols. Despite the content of the cinnamyl end-groups in lignin is relatively low, they are well involved in the pyrolysis/oxidation reactions and can have large contribution in the overall process. Therefore, the pyrolysis studies of the model compounds are necessary to clarify the detailed mechanism of lignin pyrolysis which as research evidences, occurs through the radical-chain mechanisms. Here we report the preliminary results on detection and identification of the intermediate radicals formed during the gas phase pyrolysis of the p-coumaryl (p-CMA) - one of the major model compounds, as well as the cinnamyl (CA) alcohol over the temperature range of 400-900 oC. The intermediate radicals were trapped from the gas phase using Low Temperature Matrix Isolation EPR (LTMI-EPR) technique and solid-phase spin trapping method. The solid PBN (N-t-butyl-α- phenylnitrone) and POBN (alpha-(4-Pyridyl N-oxide)-N-tert-butylnitrone) were used as spin traps. Both experimental methods confirmed the dominance of the oxygen-centered radicals in complex mixture of the radicals during the gas-phase pyrolysis of p-CMA and CA. These experimental findings are consistent with the theoretical DFT-calculations using Gaussian-03 quantum chemistry package.

LAY ABSTRACT

Radicals from the Gas-Phase Pyrolysis of Lignin Model Compounds. 1. p-Coumaryl and Cinnamyl Alcohols

Biomass pyrolysis is receiving tremendous interest as a promising renewable source of energy. As the third largest source of natural polymer after cellulose and hemicellulose and also the most abundant polymeric aromatics in nature, the conversion of lignin to biofuels is of primary importance. However, the complexity of structure makes the study of lignin pyrolysis most challenging in the field of biomass pyrolysis. A number of previous studies have shown that the most important chemistry in lignin pyrolysis is a series of free radical chain reactions with p-coumaryl (p-CMA), coniferyl and sinapyl alcohols as the three major building blocks of lignin. Moreover, cinnamyl alcohol (CA), a compound resembling p-CMA is also a major decomposition product of lignin. Therefore, the study of radicals formed during the degradation of these model compounds is essential to clarify the detailed mechanisms of lignin pyrolysis. Here we report our preliminary results on the identification of the intermediate radicals formed during the gas phase pyrolysis of p-CMA and CA over the typical temperature range of 400-900 oC. For this purposes different experimental techniques were used. The experimental findings were in accordance with the theoretical calculations.

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TECHNICAL ABSTRACT

Development of monoclonal antibodies for the real-time, quantitative detection of environmental polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants, which have been demonstrated to be carcinogenic and mutagenic. Environmental PAHs are present in complex mixtures that originate from a wide variety of natural and anthropogenic sources. To develop new antibodies that vary in their specificities to PAH mixtures, we performed a three-step screening procedure to select sensitive monoclonal antibodies (mAbs) against major classes of 3- to 5- ring PAHs. The first step employed an enzyme-linked immunosorbent assay (ELISA) screening for reactivity to the PAH-KLH immunogen and using PAH-BSA in the ELISA. The second, competitive ELISA (cELISA) employed the free PAH-tether to ascertain the relative affinity for the free PAH apart from the requisite protein carrier. Positive colonies were examined for relative specificity for 5-, 4-, 3- vs.2-ring PAHs prior to cloning. One mAb, 2G8, was selected from the pyrene immunization and another 3G10, from the phenanthrene immunization. 2G8 demonstrated uniformly high sensitivity (generic PAH recognition) against the major 3- to 5- ring PAHs. The concentrations of phenanthrene, anthracene, pyrene, chrysene and benzo(a)pyrene at 50% inhibition for 2G8 were 8.91, 5.88, 3.84, 3.84 and 1.68ug/L(ppb) respectively. It also demonstrated greater sensitivity for 3- to 5- ring PAH than a number of commercially available mAbs. In contrast an anti-phenanthrene mAb, 3G10, demonstrated much greater specificity for smaller 3-ring PAHs than for 5-ring PAHs. The results demonstrate that the screening procedure can select mAbs with high sensitivity but varying specificity to PAH mixtures to aid the evaluation of environmental samples.

LAY ABSTRACT

Development of antibody assays for the rapid analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants which possess great potential for human health problems, such as cancer and general toxicity. PAHs are found naturally in the environment but are also man-made. One can be exposed to PAHs in a number of ways, including via air, water and food. Immunoassays based on monoclonal antibodies (mAbs) are highly sensitive for the detection of PAHs and can be employed to determine concentrations in near real-time. We are developing both specific and generic anti-PAH antibodies for this purpose. In detail, a three-step screening procedure was employed for their selection. This procedure enables the relatively rapid and economic selection of various mAbs prior to the expensive and labor-intensive process of cloning and selection from hundreds of potentially inadequate mAbs. One mAb, 2G8, was selected which demonstrated a nearly uniform high sensitivity, even more than currently available commercial mAbs against major 3- to 5- ring PAHs. Alternatively 3G10 demonstrated high specificity for 3-ring over 5-ring PAHs. We believe that 2G8 could be used for rapid and sensitive detection of general PAH pollution in environmental samples, while 3G10 can enable a finer characterization of contaminant composition.

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TECHNICAL ABSTRACT

CADMIUM ASSOCIATED DYSREGULATION OF PRO-INFLAMMATORY CYTOKINES IN HUMAN PLACENTA

OBJECTIVE: To identify the associations between cadmium (Cd), zinc (Zn), and pro-inflammatory cytokine levels in human placenta.
EXPERIMENTAL PROCEEDURES: Human placental tissue samples were collected from 20 normotensive and 20 preeclamptic women. Cd and Zn metals levels were quantified by inductively coupled plasma mass spectrometry. Cytokine levels were measured using enzyme-linked immunosorbant assay and relationships between Cd and Zn metals, and cytokine levels were assessed via multivariable regression model analyses. Additionally, human JEG-3 trophoblasts were dosed for 48 hours with 2 uM Cd followed by extractions of total RNA. Extracted RNA were used in nanostring technologies immune panels to assess gene expression profiles in Cd dosed JEG-3 cells.
RESULTS: In human placenta, interactive effects were observed between Cd, Zn, and cytokine expression levels in preeclamptics that were not seen in normotensive women. In relationship to placental Cd levels, preeclamptics displayed increased levels of the pro-inflammatory cytokine IL-17 and decreased levels of the anti-inflammatory cytokine IL-1Ra. In JEG-3 cells 19 immune related genes were significantly altered in response to Cd, five are known to be associated with preeclampsia including CCL2, CDKN1A, CD64, SELE and TNFSF10.
CONCLUSIONS: Identification of Cd and Zn associated differential expression of pro-inflammatory cytokine levels may have implications for immune function in the placenta. These results identify a novel relationship between placental Cd, Zn, and cytokine protein levels in preeclamptic women.

LAY ABSTRACT

IDENTIFICATION OF AN ASSOCIATION BETWEEN CADMIUM AND IMMUNE RELATED PROTEINS LEVELS IN HUMAN PLACENTA

OBJECTIVE: To identify the associations between cadmium (Cd), zinc (Zn), and immune related protein levels in human placenta.
EXPERIMENTAL PROCEEDURES: Human placental tissue samples were collected from 20 normotensive and 20 preeclamptic women. Cd and Zn metals levels were quantified by inductively coupled plasma mass spectrometry. Immune related proteins were measured using enzyme-linked immunosorbant assay and relationships between Cd and Zn metals, and immune proteins levels were assessed via statistical software analysis. Additionally, human immortalized human placental cells (JEG-3 cells) were dosed for 48 hours with 2 uM Cd followed by extractions of total RNA. Extracted RNA were used in nanostring technologies immune panels to assess gene expression profiles in Cd dosed JEG-3 cells.
RESULTS: In human placenta, a relationship between Cd, Zn and immune related protein levels was observed in preeclamptics that were not seen in normotensive women. In relationship to placental Cd levels, preeclamptics displayed increased levels of the pro-inflammatory protein IL-17 and decreased levels of the anti-inflammatory protein IL-1Ra. In JEG-3 cells 19 immune related genes (mRNA levels) were significantly altered in response to Cd, five are known to be associated with preeclampsia including CCL2, CDKN1A, CD64, SELE and TNFSF10.
CONCLUSIONS: Identification of Cd and Zn associated differential expression of pro-inflammatory protein levels may have implications for immune function in the placenta. These results identify a novel relationship between placental Cd, Zn, and immune related protein levels in preeclamptic women.

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TECHNICAL ABSTRACT

Spatial patterns of the temporal fractal scaling of groundwater level fluctuation

We studied the fractal scaling behavior of groundwater level fluctuation for various types of aquifers in Puerto Rico using the methods of (1) detrended fluctuation analysis (DFA) to examine the monofractality and (2) wavelet transform maximum modulus (WTMM) to analyze the multifractality. The DFA results show that fractals exist in groundwater fluctuations of all the aquifers with scaling patterns that are anti-persistent ($1< \beta<1.5$; 1.32±0.12, 18 wells) or persistent ($\beta>1.5$; 1.62±0.07, 4 wells). The multifractal analysis confirmed the need to characterize these highly complex processes with multifractality, which originated from the stochastic distribution of the irregularly-shaped fluctuations. The singularity spectra of the fluctuation processes in each well were site specific. We found a general elevational effect with smaller fractal scaling coefficients in the shallower wells, except for the Northern Karst Aquifer Upper System. High spatial variability of fractal scaling of groundwater level fluctuations in the karst aquifer is due to the coupled effects of precipitation, elevation and particularly the high heterogeneous hydrogeological conditions.

LAY ABSTRACT

Spatial patterns of the changes of the long time groundwater level variations

Groundwater level reflects the balance between water input and output of an aquifer. This balance is constantly interrupted by natural phenomena such as precipitation, temperature, evaporation, and also human activities such as pumping, irrigation, and land use changes. Therefore, analyzing the water level changes is a useful proxy to understand groundwater activities in the aquifers. In this study, we analyzed the long term groundwater levels for many wells across the main island of Puerto Rico. We found that the changes of groundwater levels are related to the changes of groundwater levels in the past. This phenomenon is known as fractals. We found around 80% of the wells (18 out of 22) have anti-persistent fractals, which means that an increase in the groundwater level will be followed by a decrease in groundwater level; the remaining 4 wells showed positive persistent fractals, which means the trend (increasing or decreasing) will continue. We also found that the patterns and ability of fractal scaling behaviors differed depending on the type of aquifer. We attribute the high heterogeneity (or nonuniformity) of the karst properties as one of the main reasons causing the different patterns of fractal scaling behaviors in the groundwater level fluctuations.

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Environmental Sciences and Engineering

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TECHNICAL ABSTRACT

Investigating passive sampling strategies to monitor mercury and methylmercury in estuaries

Passive samplers have been used as powerful tool to assess “truly dissolved” fraction of contaminants in aquatic environments. Unlike conventional sampling methods, which take a single time point measurement of the total sample, passive sampling techniques assess the time-averaged, labile fraction of contaminants, providing a better assessment of the “bioavailable” fraction which is taken up by the biota. These sampling techniques have been used successfully for organic contaminants, but have not been widely applied to metals. Unlike other metals, mercury and its more toxic form, methylmercury, bioaccumulate through aquatic foodchains similar to organic contaminants. Mercury enters the base of the food chain as neutral, hydrophobic complexes. We are investigating the use of polymer and gel samplers to absorb these mercury complexes over gradients of salinity and organic carbon in estuarine waters, to investigate their ability to assess the bioavailable fraction of mercury in estuaries.

LAY ABSTRACT

Investigating sampling devices for meaningful monitoring of mercury contamination in estuaries

Mercury is a contaminant in estuaries that accumulates in marine fish, where it poses a potential health risk to humans through fish consumption. Not all mercury in estuaries is available for uptake into the aquatic food web, for example mercury that is bound tightly to sediment particles will not accumulate in fish. However, when we collect water or sediment samples to assess the extent of mercury contamination, the total mercury concentration that is measured is not representative of the concentration available to fish. In this study, we are investigating the use of sampling devices that take up the fraction of mercury that is available to marine life, to give a more meaningful measurement of contamination that may pose a human health risk.

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TECHNICAL ABSTRACT

Functional Profiling and Bioassays in Fission Yeast for Assessing Genetic Pathways Affecting Cellular Sensitivity to Chromium and other Metal Toxicants

Heavy metals and metalloids pose grave threats to human health yet their toxicity mechanisms remain poorly understood. Moreover, with few exceptions the cellular pathways that determine organismal sensitivity to heavy metals have not been systematically investigated. To address these gaps we are using a large-scale DNA sequencing platform to perform whole genome functional profiling of metal toxicants with mutant libraries of fission yeast. Remarkably, there is limited overlap with comparable screens of budding yeast, indicating divergent metal toxicant defense mechanisms and different genetic redundancies within shared pathways. One striking pattern to emerge from our Cr(VI) screen is a critical requirement for specific DNA repair and DNA damage checkpoint pathways acting in S-phase, supporting models in which chromium toxicity primarily occurs through formation of DNA adducts that disrupt DNA replication. The Mre11-Rad50-Nbs1 (MRN) protein complex, which is a “first-responder” to double-strand breaks, is particularly critical. We are further advancing our analyses by adopting a FACS-based competitive growth assay that quantifies heavy metal toxicity with exceptional sensitivity, precision and reproducibility. This liquid-based bioassay eliminates confounding heterogeneity associated with solid media assays and permits the accurate assessment of mutants that have inherent growth defects. A high throughput system to investigate genetic epistasis interactions and model human disease alleles at chromium concentrations ~ 100 ppb should significantly advance heavy metal toxicology studies. Indeed, our investigations have revealed that MRN mutations modeled on human disease alleles responsible for genome instability, neurological, developmental and immunological pathologies, and cancer predisposition, cause acute chromium sensitivity in fission yeast.

LAY ABSTRACT

Systematic and Sensitive Bioassays to Identify Pathways Determining Cellular Sensitivities to Superfund Toxicants

While industrialization has allowed for faster production and communication in our lives, the indiscriminate use of chemicals and metals and improper waste disposal has exposed us to the dangers of soil and water contamination by heavy-metals like Arsenic (As), Cadmium (Cd), Lead (Pb) and Chromium (Cr). The introduction Chromium in its soluble hexavalent form, Cr(VI), into our environments has largely been due to human activity. Cr(VI) can be taken up slowly by cells to interact with DNA forming Cr-DNA adducts. This leads to breaks in our DNA and if left unrepaired /misrepaired will prevent the faithful transfer of genetic information leading to health hazards like cancer. Although the effects of Cr(VI) toxicity is well documented, little is known about the genes that contribute towards its susceptibility. Towards this aim we have developed an assay system to quantitatively study the effects of Cr(VI) exposure across the complete genome of fission yeast. Many of the genes identified thus far have human orthologs, including some involved in genome instability diseases such as cancer. Our aim is to determine genetic pathways and functional role of genes that contribute towards greater susceptibility to Chromium. Currently our focus is on genes known to act in DNA repair pathways. We have also identified an iron-transporter gene (fep1) as hypersensitive to Cr(VI) and work is in progress to determine its role in Cr-uptake and DNA damage. This will provide more focused targets to treat heavy-metal exposure.

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TECHNICAL ABSTRACT

Development of FRET-based Displacement Assay for the Determination of Ki and koff of Environmental Toxic Chemicals Targeting Soluble Epoxide Hydrolase

Soluble epoxide hydrolase (sEH) is responsible for the metabolism of endogenously derived fatty acid oxiranes, epoxyeicosatrienoic acids (EETs) to the corresponding 1,2-diols, dihydroxyeicosatrienoic acids (DHETs). EETs are the products of arachidonic acid by cytochromes P450 epoxygenases and are key modulators involved in inflammation, pain and blood pressure. Inhibition on sEH that results in an increase of endogenous amount of EETs, has a significant effect on inflammation and blood pressure in murine model. Therefore, inhibition of sEH leads to significant effect on human health.
1,3-disubstituted urea has been reported as a general scaffold for sEH inhibitors. This scaffold is common in industrial chemicals. For example, an anti-bacterial additive in personnel care product, triclocarban, an anti-cancer drug, sorafenib, and herbicide, siduron. Therefore, human are easily exposed to these chemicals in the environment. A method to determine their potency against sEH will, thus, help us to predict the effect of these chemicals on human health through inhibition of sEH.
While Ki is an important parameter to show the binding affinity of chemicals on target enzyme, recently, several reports suggested that a kinetic parameter called koff, is as important as or more than Ki to estimate the in vivo efficacy.
Therefore, we have developed a method to rapidly determine the Ki and koff of chemicals against sEH. Here, assays of determination of Ki and koff of sEH inhibitors will be presented. Additionally, the Ki and koff of several sEH inhibitors including triclocarban and how koff affects in vivo efficacy will be showed.

LAY ABSTRACT

Develop an Assay to Measure the Potency of Environmental Toxic Chemicals against Soluble Epoxide Hydrolase

Soluble epoxide hydrolase (sEH) is an enzyme that degrades important lipid signaling molecules called epoxyeicosatrienoic acids (EETs). EETs are key modulators involved in inflammation, blood pressure, pain and angiogenesis. Therefore, inhibition of sEH which increase the level of EETs in vivo, have physiological effects on human health. Several environmental toxic chemicals, such as: triclocarban (anti-bacterial additive), sorafenib (anti-cancer drug) and siduron (herbicide) inhibit sEH. Therefore, a method to determine potency of these environmental toxic chemicals against sEH will help us predict their impact on human health. Here, we will report a new method to measure the potency of these chemicals against sEH and we will show how the measured values predict their impact on human health.

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TECHNICAL ABSTRACT

Arsenic and innate immunity: macrophage function upon arsenic exposure.

In a unique study area in Chile, our research group has reported that in utero and early-life arsenic exposures were associated with the greatest increases in young adult mortality ever associated with an early-life environmental exposure: i.e. a 7-fold increase in lung cancer and an 18-fold increase in both bladder cancer and bronchiectasis. The risks of disease and mortality remain high up to 40 years after arsenic exposures have ended. However, the mechanisms for this prolonged effect remain unknown. We hypothesize that arsenic ingestion permanently impacts immune development and increases the risks of various immune-related diseases later in life. Here we focus on macrophages, innate immune cells known to influence tumor progression and tuberculosis (TB) pathogenesis. We performed multiplex cytokine/chemokine profiling analysis on supernatant from in vitro monomethylarsonous acid (MMA3)-treated mouse bone-marrow-derived macrophages (BMDM). Our results revealed significant downregulation of various pro-inflammatory cytokines and chemokines involved in the nucleotide-binding oligomerization domain (NOD)-Like receptor, Toll-Like-receptor and peroxisome proliferator-activated receptor (PPAR) pathways, all critical in the innate immunity against TB. Lipid metabolomics experiments on these same BMDMs showed that arsenic treatment led to elevations in several pro-inflammatory and tumor-promoting signaling lipids known to play a role in tumor progression as well as the immunopathogenesis of TB. We are currently validating our findings in human macrophages and investigating how arsenic-induced immunogenic and metabolic alterations in macrophages influence TB and tumor cell pathogenicity in vitro and in vivo.
This work was supported by grant # P42ES004705 from NIEHS Superfund Research Program (M.T.S.)

LAY ABSTRACT

Arsenic may have a long-term effect on the immune system.

In a unique study area in Chile, our research group has reported that exposure of the fetus or young children to arsenic results in the greatest increase in young adult mortality ever associated with an early-life environmental exposure: notably 7 times more deaths from lung cancer and 18 times more deaths from bladder cancer and bronchiectasis (a chronic lung disease). Disease risks and mortality remain high up to 40 years after arsenic exposures have ended. However, the mechanisms for this prolonged effect remain unknown. We hypothesize that arsenic ingestion permanently impacts the development of the immune system and increases the risks of various immune-related diseases later in life. Here we focus on macrophages, immune cells known to influence the development of both tumors and tuberculosis. In experiments where we treated cultured mouse macrophages with arsenic compounds, we analyzed the secretion of small proteins necessary for communication between immune cells. Our results revealed significant decreases in secretion of various proteins that are critical in protecting against tuberculosis. When we analyzed the secretion of lipids (“fatty” molecules) by the same cultured macrophages, we found that arsenic treatment led to increased levels of several signaling lipids known to play a role in tumor development as well as the immune response against tuberculosis. We are currently validating our findings in cultured human macrophages and investigating how arsenic-induced alterations in macrophages influence the development and outcome of tumors and tuberculosis in living animals.
This work was supported by grant #P42ES004705 from NIEHS Superfund Research Program (M.T.S.)

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TECHNICAL ABSTRACT	LAY ABSTRACT

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TECHNICAL ABSTRACT

LAY ABSTRACT

Ultrasensitive Quantification of Vitamin D Metabolites using Click Chemical Derivatization and Ultra-performance Liquid Chromatography–Tandem Mass Spectrometry

Environmental contaminants may affect levels of vitamin D metabolites in our body. The first human research showed adults with higher levels of persistent organic pollutants (POPS) in their blood had lower levels of vitamin D metabolites (Yang et al. 2012). Some other papers published that chemicals may disrupt vitamin D conversion processes. It may affect the conversion of 25-hydroxyvitamin D to 1α, 25-dihydroxyvitamin D (Norris 2001, Lilienthal et al. 2000, Alvarez-Lloret et al. 2009). There might be a link between chemical exposure and the level of vitamin D metabolites. 25-Hydroxyvitamin D is the best-established indicator of vitamin D level. With its high levels (>10 ng/mL) in serum, liquid chromatography tandem mass spectrometry (LC-MS/MS) based methods could readily measure it. However, quantification of the 1α, 25-dihydroxyvitamin D by conventional LC-MS/MS methods can be highly challenging due to its extremely low circulating level (low pg/mL), short half-life (a few hours) and poor detectability. Here, liquid–liquid extraction or solid-phase extraction of vitamin D metabolites in combination a new reagent, 2-nitrosopyridine, followed by LC-MS/MS analysis was employed to provide rapid and simultaneous quantification of five metabolites (1α, 25-dihydroxyvitaminD3, 1α, 25-dihydroxyvitamin D2, 24R, 25-dihydroxyvitamin D3, 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2) at a lower limits of quantification from 25-100 pmol/mL. Such a method will help us to monitor the healthy condition of person who exposed to higher levels of air pollutants and even direct the therapeutic efforts.

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TECHNICAL ABSTRACT

The Relationship Between Lead Levels found in Maternal Serum & Umbilical Cord Blood

Lead is a known toxin in pregnancy that has several adverse maternal and fetal effects. While the degree of maternal lead exposure can be identified by obtaining a maternal blood sample, the amount of lead that the fetus is exposed to is not easily known. The goal of our study was to determine the relationship between lead levels in maternal serum and umbilical cord (UC) blood, in order to identify lead content to which fetuses and newborns are exposed. This was a prospective cohort study of pregnant women receiving care at an urban, county hospital in San Francisco. Maternal serum specimens were collected during pregnancy; UC samples were collected at delivery. Paired maternal serum and UC lead levels were obtained for 59 mother-infant pairs. Pearson's correlation coefficient was 0.93 (p=<0.001). After controlling for maternal birthplace, linear regression demonstrated that for every unit (ug/dL) increase in maternal serum lead levels, UC lead levels increased by 0.66 ug/dL (95% CI 0.59-0.72, p <0.001). Therefore, an umbilical cord blood lead level of 2.0 ug/dL could be predicted by a maternal lead level of 3.0 ug/dL. There is a strong positive correlation between lead levels found in maternal serum and UC blood, even at low levels. This suggests that maternal serum lead levels can be used as a proxy for lead content that is delivered to the fetus. This information can be used to identify which fetuses are at highest risk of lead toxicity and which newborns need screening for excess lead exposure.

LAY ABSTRACT

Understanding the Relationship Between Lead Levels found in Mother's Blood and Fetal Umbilical Cord Blood

Lead is a known toxic substance that can be found in one's surroundings, like the home or workplace. If a pregnant woman is exposed to toxic levels of lead, both her and her baby are at risk for complications such high blood pressure disorders during pregnancy, low birth weight, and problems with the baby's brain development. The goal of our study was to understand the relationship between the level of lead found in maternal blood and that found in umbilical cord blood. This is important in order to identify fetuses that may be exposed to excess lead content, so that patients can modify potential home and workplace exposures. We enrolled a group of pregnant women receiving care at an urban, county hospital in San Francisco, California into our study. We then collected their blood specimens during pregnancy and collected umbilical cord blood samples at delivery. We discovered that maternal and umbilical cord blood lead levels were positively correlated, such that for every unit (ug/dL) increase in maternal serum lead levels, umbilical cord lead levels increased by 0.66 ug/dL. This information can be used to predict how much lead the baby is exposed to by examining how much lead is in the mom's blood. This information can be used to counsel and educate patients that are at high-risk for lead exposures about the amount of lead their baby may be exposed in utero, and can be used to guide clinicians about which newborns pediatricians should screen for excess lead exposure.

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TECHNICAL ABSTRACT

TAK1 protects CCl4-mediated liver fibrosis in fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) has emerged as considerable risk factor for the steatohepatitis that lead to liver fibrosis and eventually cirrhosis. TAK1 is a MAP3K of TLRs, IL-1, TNF and TGFb receptor signalings. We previously reported that TAK1 has a protective function against hepatocyte death, inflammation and fibrosis. Our current study investigated the mechanistic role of TAK1 in steatosis-associated liver fibrosis. To elucidate the underlying mechanism, mice were subjected to high fat diet (HFD) or low fat diet (LFD) for 3 months and we injected vehicle or CCl4 injection intraperitoneally (twice a week, 0.25ul/g of BW) for last 4 weeks. HFD-fed mice were more susceptible to CCl4-induced liver fibrosis compared with LFD-fed mice as demonstrated by quantification of Sirius red-positive area. These results were supported by higher expression of profibrogenic genes such as Acta2, Col1a1, Timp1 and Tgfb1 in HFD+CCl4 group than LFD+CCl4 group. Of note, we found strong reduction of TAK1 expression in HFD+CCl4 group compared with LFD+CCl4 group. These results are consistent with the data showing reduced hepatic expression of TAK1 in genetically (ob/ob)-induced fatty liver mice. Thus, we hypothesize genetic deletion of TAK1 could replicate the features conferred by diet-induced TAK1 reduction. Indeed, hepatocyte-specific TAK1-deficient (TAK1KO) mice exhibited exacerbated CCl4-induced liver fibrosis compared with WT. Furthermore, we found that TAK1-defciency causes various pathologies including autophagy defects, aberrant mTORC1 overactivation, Smad2/3 overactivation, susceptible to cytokine-induced hepatocyte death and inhibition of PPARa. Decreased TAK1 in advanced fatty liver enhances CCl4-induced hepatocytotoxicity, resulting in increased liver inflammation and fibrosis.

LAY ABSTRACT

Fatty liver increases susceptibility to carbon tetrachloride for progression of liver fibrosis

Liver fibrosis is the excessive accumulation of scar tissue (i.e. collagen) that occurs in most types of chronic liver diseases. Advanced liver fibrosis results in liver failure, and often requires liver transplantation. Fatty liver was regarded as a mild condition, but increasing evidence suggests that fat accumulation triggers the liver to events that lead to inflammation and fibrosis. In humans, the severity of fatty liver correlates with the stage of liver fibrosis in a wide range of liver diseases. However, it remains unclear how fat accumulation can enhance liver fibrosis. We are investigating the molecular mechanisms by which fatty liver affects liver fibrosis associated with Superfund toxicant exposure (e.g. carbon tetrachloride, CCl4). CCl4 is a strong hepatotoxin that causes liver fibrosis eventually. We found that liver fibrosis of high fat-fed obese mice was more severe than that of low fat-fed lean mice. Interestingly, we observed the reduction of TAK1 expression in fatty liver. Subsequently, we tested the effect of decreased TAK1 expression to CCl4-induced liver fibrosis. Genetically engineered TAK1-deleted livers are more susceptible to liver fibrosis than wild-type liver. Collectively, these results suggest that fatty liver decreases TAK1 expression, which in turn enhances susceptibility to CCl4-induced liver toxicity and fibrosis.

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TECHNICAL ABSTRACT

Exposure to Combustion Derived Particulate Matter Suppresses Pulmonary Host Defense

Exposure to elevated levels of particulate matter (PM) is associated with adverse respiratory health in infants due to respiratory tract viral infections. However, very little is known about the associated mechanisms. Our previous studies have shown that PM impairs epithelial barrier and alters pulmonary immune response towards a regulatory phenotype and suppressed cytotoxic (Tc1) and T helper 1(Th1) responses which further increases pulmonary viral load and disease severity. To understand the mechanism of PM induced immunosuppression and increased disease severity, neonatal mice (3 days old) were acutely exposed to DCB230, a combustion derived PM (CDPM). During exposure, mice were infected with mouse adapted influenza virus and pulmonary T cell phenotypes including regulatory T cells (Tregs) were analyzed. A significant increase in pulmonary Tregs was observed after PM exposure which peaked at 10 dpe. Also, increased IL10, an immunosuppressive cytokine was observed in the lungs of PM exposed and flu infected mice at 10 dpe compared to air exposed and influenza infected controls. To further understand Treg mediated pulmonary immunosuppression, Tregs were depleted by administering anti-CD25 antibody (PC61) and infected with influenza. Significant decreases in both Th1 and Tc1 cells was observed in PM exposed and influenza infected (DCB/Flu) mice compared to air exposed and influenza infected controls, whereas Th1 and Tc1 responses were increased in Treg depleted mice exposed to PM and infected with influenza compared to DCB/Flu mice. These studies show that CDPM induced Tregs suppress adaptive T cell responses leading to increased influenza severity in infected neonatal mice.

LAY ABSTRACT

Exposure to Particulate Matter from Combustion Sources Suppresses Pulmonary Host Defense

Exposure to elevated levels of particulate matter (PM) is known to lead to poor respiratory health in infants due to respiratory tract viral infections. However, very little is known about how exposure to PM deteriorates respiratory health due to viral infections. Our previous research has shown that PM impairs respiratory barriers and suppresses protective immune responses leading to increased lung viral infection and disease severity. To understand how exposure to PM suppresses the protection against virus, we have exposed neonatal mice (3 days old) to DCB230, a PM from combustion source. During exposure, mice were infected with mouse adapted influenza virus and lung protective T cell responses were analyzed. Ten 10 days post- exposure (dpe), significant increase in regulatory T cells (Tregs) was observed in the lungs of PM exposed and flu infected mice compared to air exposed and influenza infected mice. To further understand how the suppression by Tregs cause increased viral infection in the lungs, Tregs were decreased in the lungs of PM exposed mice and infected with influenza. Significant decreases in both protective T cells was observed in PM exposed and influenza infected (DCB/Flu) mice compared to air exposed and influenza infected mice, whereas protective T cell responses were increased in mice with decreased suppressive Treg cells that were exposed to PM and infected with influenza compared to DCB/Flu mice. These studies show that exposure to PM increases Tregs and decreases protection against influenza and increases severity in infected neonatal mice.

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TECHNICAL ABSTRACT

New ways to prevent non-alcoholic steatohepatitis (NASH) progression: Defining the role of TNFR1 in de novo lipid synthesis through caspase2-mediated SREBP1 processing.

Non-alcoholic fatty liver disease (NAFLD) is a common disorder associated with obesity, and approximately 10% of NAFLD patients progress to non-alcoholic steatohepatitis (NASH), a severe and chronic liver inflammation that includes hepatocyte ballooning, fibrosis and liver damage. NASH patients are more likely to progress to HCC but the mechanisms that control NAFLD to NASH and NASH to HCC progression are poorly understood.
Our lab had recently developed a new mouse model for NASH based on feeding MUP-uPA mice, which express urokinase plasminogen activator (uPA) in their hepatocytes, with high fat diet (HFD) in which 60% of the calories come from saturated fat. Within several months these mice exhibit massive steatosis, periportal inflammation, liver damage, ballooning hepatocytes and bridging fibrosis within their hepatocytes (Nakagawa et al., 2014).
Apart from the clinical signs mentioned above, we found expression of TNF is dramatically upregulated in the liver of HFD-fed MUP-uPA mice and that TNF signaling via TNF receptor 1 (TNFR1) leads to enhanced SREBP1 processing and elevated de novo lipogenesis. Genetic ablation of Tnfr1 markedly protects MUP-uPA mice from HFD-induced liver steatosis due to inhibition of HFD-stimulated SREBP1 processing. We also found that the protease caspase 2 (Casp2) is activated in the liver of HFD-fed MUP-uPA mice and that its activation is suppressed in HFD-fed Tnfr1-/-MUP-uPA mice. Surprisingly, we have found that Casp2 is highly activated in human specimens of NASH patients but not in patients with NAFLD, leading us to postulate that TNFR1 enhances de novo lipogenesis through Casp2-mediated SREBP1 processing, resulting in NAFLD to NASH progression and a strong impetus for HCC development.

LAY ABSTRACT

New ways to prevent non-alcoholic steatohepatitis

Our study is aimed at understanding how chronic inflammation stimulates lipid accumulation in the liver, leading to progression from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH), a major causing factor of hepatocellular carcinoma (HCC). Epidemiological studies indicate that hypernutrition-induced chronic inflammation leads to NASH progression, in part, due to aberrant hepatic lipid accumulation. However, molecular mechanism by which inflammatory cytokines regulate lipid metabolism is largely unknown. In this study, we are trying to identify new molecular mechanisms that induce hepatic lipid accumulation in response to inflammatory cytokines. Also, we will try to address how these mechanisms affect NASH development and HCC incidence.

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TECHNICAL ABSTRACT

Vitamin D Receptor is a genomic gatekeeper of wound healing response in hepatic stellate cells

Liver fibrosis is a reversible wound-healing response involving TGFβ1 activation of hepatic stellate cells (HSCs). However, the molecular mechanisms governing fibrogenesis remain unclear. Here we show that vitamin D receptor (VDR) ligands inhibit HSC activation and abrogate liver fibrosis, while Vdr knockout mice spontaneously developed hepatic fibrosis. Mechanistically, we discover that VDR intersects with TGFβ-SMAD and NF-κB signaling on numerous cis-regulatory elements including those of a large set of pro-fibrotic and pro-inflammatory genes. This unique binding pattern consequentially reduces both SMAD3 and NF-κB occupancy at these sites, resulting in attenuated wound healing response in HSCs and liver fibrosis. These results thus define VDR as a genomic gatekeeper to prevent aberrant wound healing response via inhibiting both TGFβ and NF-κB signaling in HSCs, suggesting VDR ligands as a treatable mechanism to ameliorate liver fibrosis.

LAY ABSTRACT

Vitamin D attenuates liver fibrosis

Chronic liver disease and liver fibrosis/cirrhosis represent a major global health concern. In the United States, they are ranked as the eighth most common cause of mortality. Currently, no anti-fibrotic therapies for chronic liver disease have been approved by the Food and Drug Administration. Here we provide evidence that vitamin D abrogates liver fibrosis, while vitamin D receptor deficient mice spontaneously developed liver fibrosis. In addition, we discover that vitamin D reduces both pro-fibrotic and pro-inflammatory response during liver fibrosis in a genomic fashion. These results thus highlight the therapeutic potential of vitamin D for liver fibrosis.

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TECHNICAL ABSTRACT

Repeated measures of environmental exposure biomarkers during pregnancy in association with preterm birth: a case-study illustrating statistical methods for examining longitudinal predictors with a binary, non-time varying outcome

The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) Center investigates the relationship between antepartum exposure to pollutants and the associated risk of preterm birth. The goal of Project 1 is to explore the epidemiologic relationship between biomarkers of environmental exposures measured in maternal urine samples at three time points during pregnancy and the association with preterm birth. However, statistical methods for examining a time-varying exposure in association with a non time-varying outcome are poorly established and minimally utilized in environmental epidemiology studies. In the present analysis we examine 9 statistical methods that may be utilized to estimate the relationship between a longitudinal exposure and a binary, non-time varying outcome, and exemplify these methods with data from a separate study examining repeated measures of urinary phthalate metabolites during pregnancy in association with preterm birth. These methods may be useful for: 1) Examining sensitive windows of exposure; 2) Summarizing repeated measures to estimate the relationship between average exposure and an outcome; 3) Identifying acute exposures that may be relevant; and 4) Understanding the contribution of temporal patterns in exposure levels. For the PROTECT study and other projects examining a longitudinal exposure in association with a binary, non-time varying outcome, these methods may be of great utility for elucidating the complex relationships between environmental chemical exposures and preterm birth in addition to investigating biological mechanisms associated with prematurity.

LAY ABSTRACT

Statistical methods for examining the relationship between chemical exposures during pregnancy and preterm birth

The Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) Center investigates the relationship between exposure to pollutants during pregnancy and the associated risk of preterm birth. To understand these relationships, Project 1 of the Center measures indicators of mothers' chemical exposures in urine samples collected from three time points during pregnancy. Examining the relationship between multiple urinary chemical concentrations and birth outcomes is not straightforward, and previous studies using simple statistical techniques for investigating these associations may be missing important information. This study examines nine statistical methods that may be utilized to examine this type of data—some of which are simple, and some of which are quite complex. One simple method is to average the levels of chemicals measured in all three urine samples collected, and look at the relationship between those averages and preterm birth. However, a more complex method is to investigate how the change in chemical levels from the beginning to the end of pregnancy relates to prematurity. Depending on the structure of the data (e.g., how many subjects are in the study, how many samples are available, how stable the chemical measurement is in the urine sample), different methods may be more useful than others. For the PROTECT study, as well as other projects measuring an exposure at multiple time points in relation to a single outcome measure, each of these methods should be considered for data analysis. Utilizing multiple methods will improve understanding of the complex relationships between environmental chemical exposures and health outcomes.

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TECHNICAL ABSTRACT

Stable isotope dilution UPLC-MS/MS quantitation of dibenzo[def,p]chrysene adducts in a transplacental mouse model

Metabolism of dibenzo[def,p]chrysene (DBC) to the genotoxic metabolite, 11,12-dihydrodiol-13,14 epoxide (DBCDE), by P4501B1 results in the formation of covalent DNA-adducts believed to be the initiating event in DBC carcinogenesis. Our laboratory previously reported DBC to be a transplacental carcinogen resulting in lymphoblastic leukemia and lung carcinoma in murine offspring. Furthermore, DBC tissue concentration in the neonate was found to be one tenth of the maternal burden reinforcing the sensitivity of the developing fetus to environmental insults. However, the number of stable adducts formed in the neonate remains unknown. Therefore this study was designed to determine DBCDE-adduct frequency using a quantitative UPLC-MS/MS method. Pregnant B6129SF1/J dams received a single oral dose of 15mg/kg DBC on gestational day 17. Offspring were euthanized at birth and lung tissue was analyzed for adduct formation using stable isotope dilution UPLC-MS/MS. Analyses revealed adduct formation in neonate lung tissue at levels comparable to that of the maternal tissue. In addition, the (±)anti-cis-DBCDE-dA adduct was most prevalent in both neonate and dam lung tissue. We conclude that DBC or its metabolites are able to traverse the placenta and form stable adducts in neonate lung contributing to the formation of lung carcinoma after an in utero exposure. Future studies will examine other DBC target tissues, in addition to addressing DNA repair and adduct stability. Supported by CA90890, ES016465, ES007060.

LAY ABSTRACT

Measurement of DNA adducts after exposure to an environmental contaminant in the womb

Polycyclic aromatic hydrocarbons (PAHs) are a family of environmental contaminants formed through burning organic material such as fossil fuels. When humans are exposed to PAHs by skin contact, inhaling polluted air, or eating foods like barbequed meats, the PAHs become activated. Once activated, PAHs can bind to DNA to form “adducts.” These adducts have the potential to cause mutations in DNA leading to detrimental outcomes including cancer. During pregnancy, the developing fetus is also exposed to PAHs the mother encounters. Our laboratory previously reported offspring born to pregnant mice (dams) exposed to a PAH develop leukemia and lung cancer after birth. However, we do not know if or how many of these damaging adducts form in the offspring. Therefore this study was designed to examine DNA adducts in day old mice exposed to the PAH dibenzo[def,p]chrysene (DBC) during development in the womb. Using a sensitive method called ultra performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) we were able to detect adducts in lungs of one day old offspring. Our analyses reveal similar levels of adducts in dams and their offspring. We conclude DBC is activated in the developing fetus forming stable adducts. Future studies will investigate additional time points after birth to determine how long adducts remain detectable in the offspring. With this research we hope to elucidate the susceptibility of the developing fetus to PAH exposure and better understand how adduct formation leads to the development of cancer in the offspring. Supported by CA90890, ES016465, ES007060.

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TECHNICAL ABSTRACT

Safety and Health Management of Hazards Associated with Emerging Technologies

The Harvard University School of Public Health, partnering with Tulane University School of Public Health and Tropical Medicine, and Massachusetts Institute of Technology, is developing a three-tiered program of education and training in the management of hazards associated with emerging technologies.
We offer a graduate level Master of Public Health (MPH) degree and professional continuing education and distance learning programs. The overall goal is to prepare professionals and researchers in the evaluation, handling, and management of hazardous substances and conditions associated with emerging technologies including nanotechnology, energy exploration and development, drug delivery in healthcare, and sustainable remediation.
We have redesigned and updated courses in our graduate curriculum. The traditional "Industrial Ventilation" course has been refocused and retitled "Workplace Environmental Controls", addressing control of hazards associated with new technologies. We have also added case studies on emerging technologies to courses on environmental chemistry and exposure assessment, including exposures to ultrafine particles, PAHs and VOCs from 3D printers and laser surgery procedures. Trainees in the graduate program have researched hazard assessment, control and management in workplaces ranging from construction to health care. These student projects are the basis for teaching case studies in the graduate curriculum, and the continuing education and distance learning components. We are preparing a new course "OH&S Management Practices for Emerging Technologies" to develop management skills and knowledge to solve EH&S challenges of emerging technologies. This topic will also be addressed in a new 3 – 5 day executive and professional education course and a companion distance learning course.

LAY ABSTRACT

Addressing Hazards of New Technologies

Emerging technologies present new health and safety challenges in the evaluation, management, and handling of hazardous substances. New technologies such as hydraulic fracking, nanotechnology, and 3D printing pose new hazards. This project will provide education and training in methods to identify, evaluate and control these hazards through graduate level courses, continuing education and distance learning opportunities. The education programs will help further the Superfund Research Program's aim of protecting workers from the effects of hazardous substances on human health.

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TECHNICAL ABSTRACT

Trophallaxis of Food and Insecticide Lethal Load in Argentine Ants

We characterized trophallaxis between individual worker ants and examined the toxicant load in dead and live Argentine ants in a colonies exposed to two insecticides having different toxicity mechanisms. About 50% of meals with trace levels of 14C-sucrose, 14C-hydramethylnon, and 14C-fipronil were shared between single donor and recipient ants. Dead workers and queens contained significantly more hydramethylnon (122.7 and 22.4 amol/µg ant; respectively) than did live workers and queens (96.3 and 10.4 amol/µg ant; respectively), with the highest amounts in the abdomen. Dead workers had significantly more fipronil (420.3 amol/µg ant) than did live workers (208.5 amol/µg ant), but dead and live queens had equal fipronil levels (59.5 amol/µg ant versus 54.3 amol/µg ant), with the highest amounts of fipronil in the thorax of dead queens and in the head of live queens. Resurgence of polygynous ant colonies treated with hydramethylnon baits may be explained by queen survival of sublethal doses resulting from the slowing of trophallaxis throughout a colony. Bait strategies and dose levels for controlling insect pests can be based on specific toxicant behavior and trophic strategies of the entire colony. This work was performed in part under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

LAY ABSTRACT

Insecticide Transfer and Lethal Load in Argentine Ants

We measured food transfer between individual ants and the levels of two common pesticides in live and dead Argentine ants in colonies. Argentine ants are an invasive species that have displaced native ants throughout much of California. About half of ant meals and pesticides were transferred between individual ants. The pesticides studied, hydramethylnon and fipronil, poison insects in different ways. Dead workers and queens had significantly more hydramethylnon than did live workers and queens. Although dead workers had more fipronil than live workers, dead and live queens had equal fipronil levels. Live queens had most fipronil in their heads while dead queens had higher levels of fipronil in their bodies. Argentine ant colonies have multiple queens, and all must be killed to prevent resurgence of colonies. Bait strategies and low dose levels of specific pesticides can be employed to eliminate all queens before all workers die so that colonies can be eradicated. Improved bait design can reduce the concentration of pesticides used and the total amount released in the environment.

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TECHNICAL ABSTRACT

Model Light-Activated Catalysts for Pollutant Degradation

The effective elimination of toxic organic pollutants, such as polychlorinated biphenyls (PCBs), has been a major concern in the area of environmental remediation and remains an ongoing challenge. Due to adverse human health and environmental effects, the use of PCBs has been stopped and remediation methodology for existing PCB contamination has become increasingly important. One promising approach for PCB remediation is photocatalytic degradation. To this end, we have fabricated light-activated multicomponent materials as model catalysts for sustainable and efficient degradation of priority pollutants. For this, Pd nanoparticles were deposited onto Cu2O cubes to generate a composite structure. Once characterized, their hydrodehalogenation activity was studied via the reductive dechlorination of PCBs. H2 generation was observed via H2O reduction at the Cu2O surface, followed by dehalogenation at the Pd using the in situ generated H2. The Cu2O/Pd materials exhibited significant catalytic functionality for the dechlorination of PCBs. To demonstrate the versatility of this unique light-activated system, the shape and morphology of the underlying material has been varied to determine structural effects on catalysis. In this regard, Cu2O octahedra of various sizes have been synthesized to incorporate into the system in anticipation of further pollutant degradation. Their photocatalytic reactivities have been initially evaluated via the photodegradedation of model pollutants. Our work is likely to widen awareness of green and sustainable approaches in nanochemistry and nanotechnology that can contribute to environmental remediation, as well as broaden community understanding of pollutant toxic effects, thus advancing shared environmental and cultural goals.

LAY ABSTRACT

Model Catalysts for Pollutant Degradation

Elimination of toxic man-made pollutants, such as polychlorinated biphenyls (PCBs), from the environment remains an ongoing challenge. One promising approach for PCB remediation employs light as the energy source in a process called photochemistry. In photochemistry, chemical reactions proceed with the absorption of light energy by atoms or molecules. Everyday examples of photochemistry include photosynthesis and the formation of vitamin D in the body. For this, we have developed materials that absorb light as model catalysts for sustainable and efficient degradation of pollutants. Catalysts are substances that drive chemical reactions faster without undergoing any chemical change itself. Light-activated metal oxide structures were constructed and their catalytic ability was studied via the breakdown of PCBs. Hydrogen gas was produced from water using light at the surface of the metal oxide structures. Subsequently, this gas was used to drive the degradation of the PCBs. The materials showed great catalytic abilities. To demonstrate the versatility of this unique light-activated approach, the shape of the underlying material has been varied where different shapes are known to have different catalytic properties due to changes in the particle surface. Their catalytic abilities have been initially evaluated through PCB breakdown. From this research, we are showing how light can be used to speed up chemical reactions using approaches that could be used for generations.

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TECHNICAL ABSTRACT

Assessment of Pesticide Concentrations Across the Surface Water-Sediment Interface Using In Situ Solid Phase Extraction – The In Situ Sampler for Bioavailability (IS2B)

Accurate determination of porewater and bulk water concentrations of hydrophobic organic chemicals (HOCs) is essential for the estimation of bioavailable fractions of contaminant masses present in sediments and overlaying surface waters. Here, we report on the design and evaluation of a novel active sampling device, the in situ sampler for bioavailability assessment (IS2B). The IS2B enables simultaneous sampling of bulk surface water and porewater using a low-flow, 6-channel pump that delivers water to an array of solid-phase extraction (SPE) cartridges. Initial validation of the sampler was performed using five fiprole pesticides as targets. Recoveries from organic carbon-laden water (8 ppm OC), spiked with fiproles to a nominal concentration of either 1 ng/L or 10 ng/L, ranged from 82 +/- 14% to 110 +/- 18%; limits of detection ranged from 40-480 pg/L. Extraction and quantitation of total fiproles at a wastewater-impacted wetland yielded bulk surface water and porewater concentrations ranging from 9.9 +/- 4.6 ng/L to 18.1 +/- 4.6 ng/L and 9.1 +/- 3.0 ng/L to 12.6 +/- 2.1 ng/L, respectively. Detected concentrations were comparable to those determined by laboratory techniques using in-lab automated SPE. Results indicate the feasibility of using the IS2B for in situ pre-concentration of certain HOCs in lieu of or in combination with passive and conventional grab sampling techniques. The principal advantages of the new IS2B active sampler include: dual-phase sampling capability, detection limits down to the pg/L range, mitigation of sample handling losses, elimination of the need to transport either water or sediment, and customizable deployment timeframes for time-averaged sampling.

LAY ABSTRACT

Assessment of Pesticides in Rivers, Lakes, and Streams Using a New Sampling Device to Magnify Chemical Signals – The In Situ Sampler for Bioavailability (IS2B)

It is estimated that about 10% of all bodies of water are contaminated with chemicals that have adverse environmental effects. Pesticides constitute a significant proportion of these impactful chemicals, and are implicated in a number of effects such as colony collapse disorder in honeybee populations, eggshell thinning in various species of birds of prey, and androgynous development of male alligators. Adverse effects may be triggered by trace levels of pesticides like fipronil, the active ingredient of insect bait, termiticides, and flea-and-tick treatments. For risk assessment purposes, it is important to determine the mass of contaminant present in the fraction that is dissolved and readily “available” to living organisms; bioavailable fipronil may reside both in sediment as porewater and in overlaying water known as bulk water. Here, we report on the design and evaluation of a new sampling device, the in situ sampler for bioavailability assessment (IS2B). The IS2B is designed to simultaneously sample sediment porewater and bulk water in rivers, lakes, streams, and wetlands. The IS2B was demonstrated to facilitate the detection fipronil at levels much lower than conventional methods allow. The sampler was successfully tested in Arizona, where fipronil detections were linked to the discharge of treated municipal wastewater into a wetland.

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TECHNICAL ABSTRACT

Assessing advanced oxidation reactor performance using high resolution mass spectrometry

This research addresses two key challenges in applying high resolution mass spectrometry to environmental analysis: (i) developing and validating revised workflows, and (ii) maximizing the information derived from the large datasets generated. These approaches are illustrated by evaluating the performance of a novel advanced oxidation reactor with respect to contaminant destruction by photolysis and/or hydroxyl radical reactions and the attendant formation of byproducts. The pilot scale reactor was operated at different flow rates and peroxide doses under steady state conditions. Samples, spiked with 14 probe compounds selected to challenge the extraction, analysis, and treatment systems, were compared before and after treatment. Constituents were concentrated using solid phase extraction and analyzed by liquid chromatography – quadrupole time of flight (LC-QTOF) MS. Resulting data was processed to identify putative constituents not present in blank samples and aligned across all replicates in the dataset. An in-house database of ~2000 known and suspected environmental contaminants was queried, and matches were evaluated based on mass error, isotope spacing and height. Good matches that fell within the 95% prediction interval of a retention time filter were selected for MS/MS experiments. If the accurate mass of observed fragments could be explained by the suggested structure, a standard, if available, was purchased and compared. Approaches for evaluating reactor performance and byproduct formation are illustrated and provide a basis for subsequent reactor optimization.

LAY ABSTRACT

A new approach to evaluating the effectiveness of contaminant removal

Risks at Superfund sites are typically assessed, and remedial technologies are selected, based on the presence and potential removal of specified "target" constituents found in groundwater or soil. This approach may fail to identify or treat constituents not on the "target" list because they have not yet been recognized as hazardous (i.e., emerging contaminants), they are environmental degradation products of target compounds, or they are byproducts or impurities associated with the production of commercial chemicals. Ideally, reactors used to treat hazardous wastes would be evaluated for their ability to remove the widest possible range of contaminants. New equipment and data processing approaches facilitate broad, "non-target" analysis of wastes entering and leaving a treatment reactor and provide a more holistic view of reactor performance. In this research, the performance of a reactor designed to remove organic contaminants via chemical oxidation is evaluated using these broader performance measures, and their use in optimizing reactor operating conditions is illustrated.

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TECHNICAL ABSTRACT

Real-time prediction of PAH oyster contaminant loads via immunosensor analysis of sediment pore water

Lipophilic contaminants such as polycyclic aromatic hydrocarbons (PAH) concentrate in sediments and may accumulate in biota, posing a significant human health risk when consumed. Shellfish have limited ability to metabolize PAH so accumulation is primarily governed by lipid partitioning. Because measuring contaminant uptake in biota is time consuming and expensive, multi-phase models have been developed to predict contaminant bioaccumulation. However, the heterogeneity of natural habitats makes it difficult to reliably predict bioaccumulation from measured bulk sediment concentrations and properties. Ultimately, site-specific pore water measurements are needed to accurately predict contaminant bioavailability. Advances in immunosensor technology allows near real-time measurement of contaminants at sub-ppb concentrations in aqueous samples. We have used a rapid, quantitative, monoclonal antibody (mAb)-based sensor to measure PAH concentrations in sediment pore water, which, is used as a predictor of oyster concentrations. Oysters, sediment, and pore waters were collected at 18 sites in the Elizabeth River, VA including a Superfund site and others undergoing sediment remediation. PAH concentration variability on various spatial scales (meters to kilometers) was evaluated to determine the best method to predict oyster bioaccumulation. Correlations between pore water PAH measured by immunosensor (< 1 µg/L - > 800 µg/L) and oyster PAH concentrations measured by GC-MS (< 1 mg/kg - > 20 mg/kg) demonstrated that pore water was a good predictor of oyster bioaccumulation on a watershed scale. Results also demonstrated that oysters integrate exposure over a fairly large spatial scale (1 km), which can be an important factor in determining the success of phased remediation efforts.

LAY ABSTRACT

Developing a rapid assay to predict polycyclic aromatic hydrocarbon (PAH) contaminant concentrations in oysters from polluted environments

Many environmental contaminants have limited water solubility and therefore accumulate in sediments providing a long-term source to aquatic organisms. Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants, which have been demonstrated to be carcinogenic and mutagenic. Over time these contaminants may accumulate in biota, potentially posing significant human health risk when the latter are consumed. Sites with historical creosote or oil contamination often have high PAH concentrations in the sediments. Shellfish have limited ability to metabolize PAH so accumulation is governed by the concentrations in the environment and partitioning to lipid-rich tissues in these animals. Because measuring contaminant uptake in biota is time consuming and expensive, new methods are needed to accurately predict uptake of PAH by shellfish to evaluate risk to consumers and guide remediation efforts to clean up contaminated sediment sites. Advances in new immunosensor technology now allows near real-time measurement of contaminants at very low concentrations in aqueous samples. We have used an antibody-based sensor to measure PAH concentrations in sediment pore water as a predictor of oyster tissue concentrations. PAH concentrations measured by the immunosensor were found to be a good predictor of tissue burdens in the oysters. Using this technology evaluation of the effectiveness of sediment remediation efforts can be assessed and used to guide future remediation plans.

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TECHNICAL ABSTRACT

FLAXSEED AND ITS LIGNAN COMPONENT PROTECT FROM ASBESTOS-INDUCED INFLAMMATION IN MICE

Background: Malignant mesothelioma (MM) is a highly lethal form of thoracic cancer with high mortality and poor treatment options. Development of MM has been linked directly to exposure to asbestos fibers, but with a long latency period. Chronic inflammation and oxidative tissue damage caused by persistent asbestos fibers has been linked to asbestos carcinogenesis. Whole grain flaxseed (FS) has known antioxidant, anti-inflammatory and cancer chemopreventive properties. Rationale: As a prelude to future chemoprevention studies, we tested the ability of oral FS and its lignan component, (FLC) to prevent acute asbestos-induced inflammation and inflammatory cytokine release in MM-prone Nf2+/mut;Cdkn2a+/mut mice which develop an accelerated form of MM when exposed to asbestos. Methods: Mice were given a single ip bolus of 400 ug of crocidolite asbestos. They were then placed on 10% FS or 10% FLC supplemented diets 1 day prior (-1) to or after (+1) asbestos instillation and evaluated 3 days after injection of asbestos for abdominal inflammation and proinflammatory cytokine release. Results: Systemic levels of flaxseed lignan metabolites were comparable to those in other mouse models where FS was shown to be an effective chemopreventive agent. Both FS and FLC blunted acute abdominal inflammation induced by asbestos by decreasing inflammatory cell numbers and TNF-alpha and IL1-beta levels in peritoneal lavage fluid. Conclusions: Our findings suggest that FS and its lignan component appear to reduce short-term asbestos-induced inflammation and may thus prove to be promising dietary agents in the chemoprevention of MM.
Funding: Supported by NIH grants P42ES027320 and R03CA180548

LAY ABSTRACT

FLAXSEED AND ITS LIGNAN COMPONENT PROTECT FROM ASBESTOS-INDUCED INFLAMMATION IN MICE

Background: Malignant mesothelioma (MM) is a highly lethal form of thoracic cancer with high mortality and poor treatment options. Development of MM has been linked directly to exposure to asbestos fibers, but with a long latency period. Chronic inflammation and oxidative tissue damage caused by persistent asbestos fibers has been linked to asbestos carcinogenesis. Whole grain flaxseed (FS) has known antioxidant, anti-inflammatory and cancer chemopreventive properties. Rationale: As a prelude to future chemoprevention studies, we tested the ability of oral FS and its lignan component, (FLC) to prevent acute asbestos-induced inflammation and inflammatory cytokine release in MM-prone Nf2+/mut;Cdkn2a+/mut mice which develop an accelerated form of MM when exposed to asbestos. Methods: Mice were given a single ip bolus of 400 ug of crocidolite asbestos. They were then placed on 10% FS or 10% FLC supplemented diets 1 day prior (-1) to or after (+1) asbestos instillation and evaluated 3 days after injection of asbestos for abdominal inflammation and proinflammatory cytokine release. Results: Systemic levels of flaxseed lignan metabolites were comparable to those in other mouse models where FS was shown to be an effective chemopreventive agent. Both FS and FLC blunted acute abdominal inflammation induced by asbestos by decreasing inflammatory cell numbers and TNF-alpha and IL1-beta levels in peritoneal lavage fluid. Conclusions: Our findings suggest that FS and its lignan component appear to reduce short-term asbestos-induced inflammation and may thus prove to be promising dietary agents in the chemoprevention of MM.
Funding: Supported by NIH grants P42ES027320 and R03CA180548

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TECHNICAL ABSTRACT

Functional Genetic Screen in Human Haploid Cells to Identify Genes Involved in Susceptibility to Chemical Exposure

Established functional genetic screening systems, such as yeast and RNA interference, have been successfully applied to study susceptibility to chemical toxicity but they are limited by relevance to human and incomplete knockdown, respectively. The KBM7 human haploid cell screening approach overcame these limitations but the original protocol was lengthy and inefficient. We recently optimized a screening platform using a KBM7 mutant cell library (KBM7-Mu). Utilizing a semi-solid, rather than the original liquid, medium, we: 1) enabled the simultaneous screening and generation of distinct colonies from individual mutant cells resistant to a chemical of interest; 2) shortened the entire screening process by approximately 3 weeks; and 3) decreased the rate of false positives. Using this improved approach, we identified a dozen human genes that confer resistance to formaldehyde (FA), a human leukemogen. Three genes, LPR5 (low-density lipoprotein receptor-related protein 5), GOT1 (glutamic-oxaloacetic transaminase 1) and M1AP (meiosis 1 associated protein) were confirmed in two independent screening experiments. Further, LRP5, GOT1 and M1AP mutant KBM7 cells showed significant resistance to FA-induced toxicity compared to wild-type cells (KBM7-wt). Quantitative RT-PCR and western blotting confirmed the knockdown of transcription and knockout of translation of the LRP5 gene in LRP5-mutant KBM7 cells. Our findings suggest that LPR5, GOT, M1AP are involved in susceptibility to FA toxicity and they further demonstrate the broad applicability of this optimized screening approach to discover novel susceptibility genes and toxicity mechanisms associated with chemical exposures. (Supported by NIEHS P42ES004705 to MTS and R01ES017452 to LZ)

LAY ABSTRACT

Finding the genes which confer sensitivity to formaldehyde and other toxic chemicals

We know that people differ in their sensitivity to the toxic effects of chemicals. However, we do not know which individuals will be more susceptible. Many of the biological pathways leading to toxic effects involve genes, which are part of the body's DNA. Genes produce proteins that can start or stop biological processes relevant to toxic effects. This is called "gene expression" and it varies a lot. This variation can be because of differences in the genes themselves or in diet and chemical exposures, among other factors. These differences can contribute to greater susceptibility to chemical exposures. We have limited information about which genes contribute to greater susceptibility to toxic chemicals. To determine this, we improved a method using human cells to screen for genes that confer susceptibility to chemical agents. The human "cell line" employed was modified to have one set of genes, rather than the usual two sets (one from the mother and one from the father). We screened for genes that contribute to susceptibility to formaldehyde, which is in resins and adhesives added to everything from paper napkins to carpeting. Formaldehyde is an irritant and causes cancer in humans and laboratory animals. We identified 11 genes as potentially causing susceptibility to formaldehyde. We confirmed two of these in additional experiments. The genes are GOT1 (Glutamic-oxaloacetic transaminase 1) and M1AP (Meiosis 1 Associated Protein). We don't know how these genes confer susceptibility. Additional studies in human populations and in cell cultures are needed to discover how this works.

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TECHNICAL ABSTRACT

Prenatal arsenic exposure and the epigenome: Identifying sites of 5-methyl cytosine alterations that predict functional changes in gene expression in newborn cord blood and subsequent birth outcomes

Prenatal exposure to inorganic arsenic (iAs) is detrimental to the health of newborns and increases the risk of disease development later in life. Here we examined a subset of newborn cord blood leukocyte samples collected from subjects enrolled in the Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Gómez Palacio, Mexico who were exposed to a range of drinking water arsenic concentrations (0.456-236 ug/L). Changes in iAs-associated DNA 5-methyl cytosine methylation were assessed across 424,935 CpG sites representing 18,761 genes and compared to corresponding mRNA expression levels and birth outcomes. In the context of arsenic exposure, a total of 2,919 genes were identified with iAs-associated differences in DNA methylation. Site-specific analyses identified DNA methylation changes that were most predictive of gene expression levels where CpG methylation within CpG islands positioned within the first exon, the 5' untranslated region and 200bp upstream of the transcription start site yielded the most significant association with gene expression levels. A set of 16 genes was identified with correlated iAs-associated changes in DNA methylation and mRNA expression and all were highly enriched for binding sites of the early growth response (EGR) and CCCTC-binding factor (CTCF) transcription factors. Furthermore, DNA methylation levels of seven of these genes including imprinted gene KCNQ1 were associated with differences in birth outcomes including gestational age and head circumference. These data highlight the complex interplay between DNA methylation, functional changes in gene expression and health outcomes and underscore the need for functional analyses coupled to epigenetic assessments.

LAY ABSTRACT

Prenatal arsenic and the epigenome

Exposure to inorganic arsenic (iAs) during pregnancy can harm newborns and increases whether individuals are diseased later in life. Here we examined a subset of newborn cord blood samples collected from subjects enrolled in the Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Gómez Palacio, Mexico who were exposed to a range of drinking water arsenic concentrations (0.456-236 ug/L). Changes in iAs-associated tagging of methyl groups onto cytosine was assessed across 424,935 CpG sites representing 18,761 genes and compared to corresponding mRNA expression levels and birth outcomes. In the context of arsenic exposure, a total of 2,919 genes were identified with iAs-associated differences in DNA methylation. Site-specific analyses identified DNA methylation changes that were most predictive of gene expression levels Changes in the methylation tagging of seven of these genes including imprinted gene KCNQ1 were associated with differences in birth outcomes including gestational age and head circumference. These data support complex relationships between DNA methylation, functional changes in gene expression and health outcomes and underscore the need for functional analyses coupled to epigenetic assessments.

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TECHNICAL ABSTRACT

Environmentally Persistent Free Radicals Inhibit CYP2B4 by Disruption of NADPH-cytochrome P450 Reductase-CYP2B4 Complex Formation

Combustion of industrial wastes generates particulate matter (PM) that can affect human health, with the most harmful effects being associated with ultrafine particles (<2.5 μm). Combustion of aromatic hydrocarbons (particularly halogenated varieties) with redox-active metals generates ultrafine particles containing air-stable, environmentally persistent free radicals (EPFRs). Exposure to EPFRs has been shown to negatively influence pulmonary and cardiovascular functions. Cytochromes P450 (P450/CYP) are endoplasmic reticulum resident proteins that are responsible for the metabolism and elimination of foreign compounds. Model EPFRs generated from heating silica containing 5% copper oxide (CuO-Si) with either dicholorobenzene (DCB230) or 2-monochlorophenol (MCP230) above 230 C inhibited several P450s and inhibited metabolism noncompetitively. Inhibition by EPFRs was specific for P450s and did not affect their redox partner, NADPH-cytochrome P450 reductase (CPR) or HO-1, which also relies on electrons from CPR. In this study, the specific mechanism of P450 inhibition by MCP230 was examined using a purified system with CYP2B4. MCP230 inhibited the CYP2B4-mediated metabolism of 7-ethoxy-4-trifluoromethylcoumarin (7EFC) greater than 10-fold more potently than non-EPFR controls (MCP50, CuO-Si, and silica). All of the nanoparticles inhibited CYP2B4-mediated metabolism noncompetitively with respect to substrate and the Ki constants for inhibition ranged from 0.024 mg/mL for MCP230 to >0.5 mg/mL for MCP50. When CYP2B4-mediated metabolism of 7EFC was measured as a function of the CPR concentration, the mechanism of inhibition was competitive. Thus, EPFRs likely inhibit substrate metabolism by disruption of the CPR•CYP2B4 complex, and in turn, the delivery of electrons needed for catalysis. (Supported by P42 ES013648)

LAY ABSTRACT

Airborne Particulates Inhibit Enzymes Responsible for the Elimination of Foreign Compounds

Combustion of industrial wastes generates particles that can affect human health, with the most harmful effects being associated with the smallest particles (<2.5 μm). When Superfund wastes are burned they produce these ultrafine particles that generate damaging reactive oxygen. These particles are stable in the environment (and are known as environmentally persistent free radicals (EPFRs)), and can damage lung and heart function once they enter the organism. Cytochromes P450 (P450) are enzymes that are responsible for the breakdown and elimination of drugs and foreign compounds. Our data shows that EPFRs inhibit the ability of P450 to eliminate drugs and other foreign compounds. The inhibition of one particular P450 enzyme was studied. The data showed that the EPFRs did not block the region of the P450 where the drug/foreign compound binds. The EPFR appeared to lay on the surface of the P450 enzyme, blocking the ability of the P450 to combine with another protein that supplied the energy necessary for drug metabolism. (Supported by P42 ES013648)

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TECHNICAL ABSTRACT

LAY ABSTRACT

Overview of the Midwest Emerging Technologies Public Health and Safety Training (METPHAST) Program

The long-term goal of the Midwest Emerging Technologies Public Health and Safety Training (METPHAST) Program is to ensure that emerging technologies grow without causing illness or injury to workers and the public. The program's central objective for the current three-year funding period is to develop a comprehensive array of focused, web-based modules about nanotechnology health and safety that can be used flexibly by instructors to create academic courses, continuing education initiatives, and individual lessons that serve the unique needs of different learners. This objective is being met by developing 20 one-hour, web-based modules and supplemental hands-on activities to train professionals to work safely with engineered nanomaterials. We will incorporate the modules and hands-on activities into two academic courses for university-level students. The first course will introduce students in science and engineering to occupational health and safety. The second will provide in-depth training to occupational health and safety students and science and engineering students regarding nanotechnology health and safety. The modules will also be offered for continuing education credit for professionals who need on-going training for certification maintenance. Finally, the materials from each module will be made freely available to instructors at high schools and two-year colleges through Nano-Link, a web site that disseminates web-based nanotechnology training materials, so that the instructors can develop lessons that address the needs of their students. The result of the currently-funded efforts of the METPHAST Program will be a workforce better prepared to anticipate, recognize, evaluate, and control nanotechnology-related hazards.

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TECHNICAL ABSTRACT

Novel inhaled metal oxide nanoparticles advance understanding of dosimetry in the adult and developing respiratory system

There is growing interest in the fate of nanoparticles (NP) in the body due to their use in medicine and consumer products. To enable detection of particle deposition with high sensitivity and low background, we developed a spray pyrolysis synthesis method that generates an aerosol of luminescent europium-doped gadolinium oxide (Eu:Gd2O3) NP. We exposed neonatal (7days old), juvenile (21 days old) and adult (13 weeks of age) male rats using a one-hour nose-only exposure to aerosolized NP with a mean particle size of 35nm and a concentration of 338 ug/m3. Control animals were sham exposed. Exposures were within acceptable limits of carbon monoxide, nitrogen dioxide and nitrogen oxide. Rat respiratory tract tissue was taken immediately following exposure and 24hrs post exposure. Lung tissue from neonatal and adult rat lungs were within the limit of detection of ICP-MS at all time points. To determine whether this approach can measure translocation of particles from the initial site of deposition in the lung, we measured the levels of NP in brain. Nose to brain transport of NP has been reported previously for particles in this size range but is thought to represent a very small percentage of the deposited dose. The limit of detection was sufficiently sensitive to detect particles that translocate to brain tissue and this translocation occurred as soon as 1 hr following the beginning of exposure in neonatal animals. We conclude that this new method can be used to track NP deposition and particle translocation with very high sensitivity. Support from NIEHS ES04699 and ES020127

LAY ABSTRACT

Understanding where very small particles go when inhaled

Air pollution, consumer products and some medicines, contain particles that are very small called nanoparticles. Nanoparticles can have health effects, especially when inhaled. When nanoparticles are inhaled they deposit in the lungs and sometimes can move from the lungs to other organs. Movement of nanoparticles from the lungs is called translocation. Our understanding of where nanoparticles deposit and translocate in the body is hampered by our inability to track nanoparticles. We developed a new type of nanoparticle that can be inhaled and tracked. This nanoparticle contains two elements: europium (Eu) and gadolinium (Gd) that are unique and not normally found in the body. This lets us measure the particle in organs. In this study, we found that we can also use these particles to measure translocation from the respiratory tract to the brain. Recent studies have shown that immature lungs and brains, such as are found in children and young animals, respond to particles differently. We found that we can also use our new Eu:Gd particles to track where particles go, even into the brain, of very young animals. We need to understand where particles go and how they may accumulate over time so that we can understand how exposures to NP are related to health effects, especially in susceptible populations, such as the very young. We hope to use Eu:Gd particles and the exposure system we developed to understand how air pollution affects the lung and how medicines are deposited differently in young vs old subjects.

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TECHNICAL ABSTRACT

The role of epoxy-fatty acids in carbon tetrachloride-induced liver fibrosis

Liver fibrosis is a pathological condition in which chronic inflammation and changes to the extracellular matrix lead to alterations in hepatic tissue architecture and functional degradation of the liver. Liver fibrosis is triggered by a number of environmental chemicals, including carbon tetrachloride (CCl4) as well as occupational exposure to vinyl chloride and other halogenated solvents. Because inhibitors of the soluble epoxide hydrolase (sEH) have been shown to reduce fibrosis in the heart, pancreas and kidney in several disease models, we have assessed the effect of a sEH inhibitor, 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), on the development of fibrosis in a carbon tetrachloride (CCl4)-induced mouse model by monitoring changes in the inflammatory response, matrix remolding and endoplasmic reticulum stress. Collagen deposition in the liver was increased five-fold in the CCl4-treated group, and this was returned to control levels by TPPU treatment. Hepatic expression of Col1a2 and 3a1mRNA was increased over fifteen-fold in the CCl4-treated group relative to the control group, and this increase was reduced by 50% by TPPU treatment. Endoplasmic reticulum stress observed in the livers of CCl4-treated animals was attenuated by TPPU treatment. Taken together, these data indicate that the sEH may play an important role in the development of hepatic fibrosis induced by CCl4 and that environmental factors that modulate sEH expression (such as PPAR agonists) or the P450-dependent production of its epoxy fatty acid substrates (such as dioxin and arsenate) will influence the development of liver fibrosis.

LAY ABSTRACT

Lipid signals and liver fibrosis

Liver fibrosis is a pathological condition in which processes normally involved in repair of the tissue have gone awry and continue to make damaging changes to the liver's structure. In cases of exposure to certain environmental chemicals such as carbon tetrachloride, the liver is first injured by the chemical, triggering the repair process. If exposure continues, the switch from healthy repair to pathological fibrosis occurs and can lead to liver failure. We have investigated the role of signaling molecules called epoxy-fatty acids in physiological processes such as the maintenance of blood pressure, inflammation, and fibrosis of the heart and kidney. By blocking an enzyme called soluble epoxide hydrolase that degrades these epoxy-fatty acid, we are able to boost their signal, which has beneficial effects in diseased tissues, reducing both inflammation and fibrosis. In our study, by blocking soluble epoxide hydrolase, we were able to reduce the amount of fibrosis in the livers of mice treated with carbon tetrachloride. This study demonstrates a link between epoxy fatty acids levels and fibrosis as well as one potential benefit of blocking their degradation by inhibiting soluble epoxide hydrolase.

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TECHNICAL ABSTRACT

Discovery of Xenobiotics Associated with Preterm Birth

Preterm birth is a major health problem, and its incidence, which has risen significantly during a recent span of years, is especially high in Puerto Rico, an island with many Superfund sites. Evidence has been accumulating that environmental chemical exposure may contribute to preterm birth. We are analyzing the urinary metabolome (including the exposome) of pregnant women in Puerto Rico, and also the placental DNA adductome and metabolome, seeking to discover biomarkers (bystander or causative) for preterm birth. Here we present our: (1) procedure for collecting a large volume of urine (accumulated first voids are collected over a week by the woman at home to give an average exposure, and provide a large sample to increase signal/noise, enable analysis in different ways, and facilitate identification of mass spectral features that correlate with preterm birth); (2) new technique (porous solid phase extraction paddle, consisting of a reversed stirring tea bag containing a cocktail of solid phase extraction particles) for practical and reliable extraction and subsequent archiving the metabolome from a large urine sample at a remote location; (3) new method providing a broader assessment of the urinary sulfate/glucuronate conjugateome, based on analysis by PEP/LC-MALDI-TOF/TOF-MS; yielding peak patterns with significant variation among pregnant women (many environmentally-unfriendly compounds can be anticipated to end up like this in urine); and (4) DNA adduct detection by LC-MS/MS at the amol level (to facilitate measurement of the DNA adductome in placenta).
Funded by NIEHS Grant P42ES017198.

LAY ABSTRACT

Do Environmental Chemicals Contribute to Preterm Birth?

Preterm birth is a major burden on society ($26 billion per year in the US, also reflecting significant morbidity and mortality), and there has been a steep rise in its incidence in recent years. Clearly the environment is the cause, when the environment is defined in the broadest sense. The only good way to fix the environment is to find out what is bad about it for preterm birth. In this project, we are making the assumption that one or more chemical pollutants are driving the high level of preterm births. We have chosen to focus on pregnancies in Puerto Rico since the incidence of preterm birth there is unusually high, and this island also has many Superfund pollution sites. Our approach is to collect urine from pregnant women and look for chemicals that are more abundant in women who go on to have a preterm birth. We are making no assumptions as to what the chemicals are: this is a “chemical discovery” project, analogous to forensic investigations seeking evidence broadly to help solve a crime. Method development is a part of the project, since current tools for chemical discovery analysis need to be improved. Funded by NIEHS Grant P42ES017198.

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TECHNICAL ABSTRACT

EPIDEMIOLOGIC ANALYSIS OF THE EFFECT OF MIXTURES: APPLICATION OF A NEW METHOD TO A STUDY OF ORGANOCHLORINES AND ADHD.

The health effects of exposure to mixtures is increasingly important for epidemiology, toxicology and risk assessment. Conventional epidemiologic methods are either primarily descriptive (e.g., effect measure modification) or employ a formal interaction analysis using a risk difference scale. We propose a method that borrows from spatial epidemiology and toxicology. For a mixture with two major components, we construct the smoothed joint response surface, plotting outcome on the z- axis vs. the two exposures on the x and y axes. Generalized additive models are ideal for this application because they smooth data of different outcome types while adjusting for covariates. We project the resulting surface onto the x-y plane and shade to show outcome magnitude. A toxicological interpretation can be added by drawing the contours. The shape of the contours, called isoboles, provide information about toxicological interaction and additivity. Compounds with similar modes of action will have contours that are negatively sloped straight lines if they are dose-additive. We demonstrated the proposed method using synthetic data, and then applied it to a previous conventional epidemiologic analysis of prenatal organochlorine exposure and attention deficit hyperactivity disorder (ADHD)-associated behaviors in school-aged children. The two exposures of interest were umbilical cord serum concentrations of DDE and PCBs. PCBs were highly correlated with each other so we used a summary measure. The outcome was the Conners' Rating Scale for Teachers ADHD Index. While the outcome-exposure maps suggested effects by both compounds and possible effect measure modification, the contours did not indicate dose addition.

LAY ABSTRACT

METHODS FOR UNDERSTANDING HEALTH RISKS OF EXPOSURE TO MULTIPLE CHEMICALS: "MAPPING" RISK OF ADHD FOR DIFFERENT COMBINATIONS OF EXPOSURE

Scientists typically study the health effects of toxic chemicals one at a time. But we are exposed to many chemicals at once. In addition to being an important scientific question, understanding the health effect of exposure to multiple chemicals ("mixtures") is critical when trying to understand the health risk of living near a hazardous waste site. Thinking about the potential interactions between drugs is also an important job of a pharmacist. We have been adapting ideas from pharmacy and toxicology (studies of toxic chemicals) to studies of people as they are exposed in their daily lives (environmental epidemiology). For example, suppose you want to understand the combined effect of two exposures. Imagine that you make a "map" with exposure to one chemical increasing from East to West and exposure to the other chemical increasing from South to North. The height of the map at any location shows the risk of disease at that point. If you were ever a Girl or Boy Scout, you may have seen topographic maps that show elevation: the contour lines connect points with the same elevation. We do the same thing in our chemical maps, but the contours connect points with equal health risk. Remarkably, the shape of the contour lines tells us about the kinds of interactions between chemicals that might be happening. We demonstrate this method here using data from a study examining associations between two environmental chemicals and behaviors associated with ADHD in school age children.

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TECHNICAL ABSTRACT

Research Translation and Community Engagement at Michigan State University: an Evolving Partnership with a Dioxin Contaminated Community.

The MSU-SRP conducts and translates research addressing fundamental questions relevant to our understanding of the molecular mechanisms by which dioxin and dioxin-like compounds contribute to human diseases, as well as the environmental fate of these compounds. The RTC works closely with the CEC to engage the Midland community in Michigan, which is affected by some of the highest dioxin concentrations in the world. One strategy was to reach out to this affected community via a high school curriculum on environmental health developed with the support and collaboration of teachers. This interaction has been evolving beyond curriculum development and into the development of a community-based participatory research project (CBPR). A primary goal of the project was to discover a way to give Midland high-school students a hands-on learning experience. At the same time, project-5 lead, Gerben Zylstra, expressed that the sediments of this community are a potential resource of microorganisms capable of biodegrading halogenated compounds, such as dioxin, but his group had only a limited ability to sample representative sediments. A breakthrough came when the team realized both issues could be addressed with one synergistic project: the involvement of the high-school students in sediment sampling. To locate hot spots for microbial activity for dehalogenation, Project-4 lead Syed Hashsham has developed a handheld field analyzer for identifying dehalogenating genes in microbial populations, and will loan it to the community. Our CEC will involve students in the design and assessment of their learning throughout the CBPR.

LAY ABSTRACT

A Partnership between the Michigan State University Super Fund Research Program and the Community of Midland, MI to Address Local Environmental Contamination Issues

The Michigan State University-Super Fund Research Program Center (MSU-SRP) conducts and translates research addressing fundamental questions relevant to our understanding of the molecular mechanisms by which dioxin and dioxin-like compounds contribute to human diseases, as well as the environmental fate of these compounds. The MSU-SRP Research Translation Core works closely with the MSU-SRP Community Engagement Core to engage the Midland community in Michigan, which is affected by some of the highest dioxin concentrations in the world. Our interaction with the Midland community has led to the initiation of a community-based participatory research project (CBPR) where students, teachers and parents will begin a project with potential outcomes that will help the research investigators of our center to identify potential microorganism that can potentially degrade dioxin and dioxin-like compounds. Our research team will use such organisms to understand how to optimize the conditions by which these microorganisms can decontaminate soil sediments that can ultimately lead to better clean-up options for this affected community.

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TECHNICAL ABSTRACT

Numerical Literacy, Influence Networks, and Stigma—Three Dimensions for Understanding Community Disengagement and Decision Making

Exposure to environmental pollution brings long-term health consequences to human beings of all ages. Efforts made by community residents, government agencies, and environmental groups and partnership among them are essential to contamination clean-up. Research in this project will provide answers to two important questions—the extent that residents living along the Tittabawassee River in Michigan are engaged in decisions about cleanup, and the underlying factors that affect their levels of engagement. To answer these two questions, researchers will take three approaches: (1) understanding what types of numerical information do community residents comprehend and prefer, (2) mapping out influential sources of advice accessed by community residents about dioxin pollution and cleanup, and (3) investigating the extent to which community residents feel stigmatized from living on contaminated land and how such feelings affect their engagement in contamination cleanup efforts.

The Community Engagement Cores (CEC), in partnership with the Research Translation Cores (RTC) will conduct in-depth interviews (Spring ~ Summer 2015) and survey local community residents (Fall 2015 ~ 2016) to understand the level of engagement and how the residents are involved in the decision making process, and assess the effects of numeracy, influence network and environmental stigma on their levels of engagement and decision making. The Community Advisory Group and local environmental groups will participate in the process of interview protocol and survey development, and will be debriefed about research results and the results will be used to inform future efforts in designing community education materials and community outreach activities.

LAY ABSTRACT

Finding Critical Factors that Affect Community Engagement in Dioxin Contamination Cleanup Decision Making

The Community Engagement Cores in partnership with the Research Translation Cores at Michigan State University attempt to understand the levels of community engagement in the dioxin contamination cleanup decision making among the Midland community residents in Michigan, and to investigate factors that influence the levels of community engagement. In the first phase, the researchers will interview and survey local residents to assess their current levels of knowledge about dioxin contamination and cleanup and their engagement in the contamination cleanup process. Three potential influencing variables are per-identified--influence network (i.e., who are the influential individuals in the community in the context of dioxin contamination and cleanup), numerical literacy (i.e., the extent to which people understand the numerical presentations given by scientists, technicians, and educators), and perceived environmental stigma (i.e., the extent to which people feel discriminated for living in a polluted area). The researchers will assess the individual and combined effects of the three factors on community engagement. The study results will be shared with important stakeholders in the community including the Community Advisory Group and environmental groups to inform future development of community education material and outreach events.

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TECHNICAL ABSTRACT

The Penn Interdisciplinary Superfund Research Training Program

The Penn Interdisciplinary Superfund Research Training Program provides cross-training in environmental science (ES) and environmental health science (EHS) to ensure that pre- and post-doctoral fellows who wish to conduct research in Penn-SRP laboratories have a sufficient knowledge base to understand the hazards of Superfund waste sites and use this knowledge to help remediate these sites and their ensuing health effects. The unique interdisciplinary training program marries the disciplines of ES with EHS. In our curriculum all trainees take a new course: ”Introduction to Superfund Sites and Health Effects of Hazardous Waste” which includes field trips to several Superfund sites in PA. ES students then take a toxicology curriculum and conduct rotations in the biomedical projects of the SRP. EHS students take ES courses and are required to conduct either field-work at a Superfund Site or complete a Capstone project. The program offers extraordinary experiences for those interested in the broader concept of translational science. Thus our program offers training experiences, through the Community Engagement Core (CEC) and the Research Translational Core (RTC) of the Penn SRP Center. The CEC teaches skills in risk communication. The RTC offers training options in the Center of Technology Transfer Fellows Program, for those interested in disclosures of invention and how to protect intellectual property; and offers internships at the US EPA in Washington through a memorandum of understanding. Trainees would be competitive for employment in academia, industry or government setting (e.g. EPA, ATSDR and CDC). Supported by P42-ES023720.

LAY ABSTRACT

Training a Superfund Hazardous Waste Workforce

The clean-up of Superfund hazardous waste sites requires a trained worked-force that is skilled in remediation strategies, effects of the waste on the ecosystem, and possible adverse health effects. Why it may not be possible to have the same level of skill in environmental sciences, biology and biomedicine, an appreciation of the application of all disciplines to the effective cleanup of these sites is deemed essential. Superfund hazardous waste sites are often located in communities so that bidirectional communication of ongoing research and how this may meet the needs of the community is a critical component of clean-up efforts. Often scientists are not skilled in the necessary communication skills to effectively translate their findings to affect decision making. Scientists also may lack knowledge on how to take a finding and translate this into an invention for commercialization. The Penn-SRP interdisciplinary training core addresses these unmet needs by providing cross-training in these disparate disciplines. Environmental scientists and biologists receive training in environmental health sciences and vice-versa. In addition, the Community Engagement Core provides training experiences in risk communication, and the Research Translation Core provides training experiences in technology transfer.

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TECHNICAL ABSTRACT

Direct Measurement of Estrogenic and Androgenic Compounds in Human Plasma Using a Cell-based Reporter Gene Assay

Over 230,000 cases of breast cancer are diagnosed annually in US women and approximately 40,000 die of the disease each year. Progress has been made in identifying risk factors strongly related to breast cancer risk, including plasma steroid hormone (estradiol and testosterone) levels. However, additional risk factors likely exist, such as environmental chemicals that act on estrogenic and androgenic pathways. To identify novel chemicals with endocrine disrupting activity, we have refined methods for the assessment of total activity against estrogen receptors (ER) and androgen receptors (AR) in human plasma. Bioassays using T47D-kbluc and MDA-kb2 cell lines were optimized to measure ER and AR activation, respectively, in archived plasma samples of 90 Mexican American women from the San Francisco Bay Area Breast Cancer Study (SFBCS). As expected, we found a statistically significant difference in ER activity levels between pre-menopausal (mean=498pM) and post-menopausal (mean=125pM) women (p=0.001). There was no statistical difference in AR activity between 64 samples of pre-menopausal and post-menopausal women. There was also no statistically significant difference (p=0.12) between breast cancer cases (n=15) and controls (n=75) in part due to our small sample size. Identifying subjects with widely differing levels of ER and AR activity (after subtraction of the natural steroid hormone component) is possible by measuring estradiol and testosterone levels simultaneously. This should provide a rapid, agnostic, sensitive and cost effective method for identifying environmental compounds with estrogenic and androgenic potential.

LAY ABSTRACT

Measuring Hormones and Chemical Mimics In Human Plasma Using A Cell-based Assay

In the United States, breast cancer is one of the leading causes of death among women. Apart from known risk factors such as age, genetics, bodyweight, and ethnicity, other risk factors seem to play a pivotal role. Exposure to chemicals may lead to abnormal changes in breast cells and cancer development by altering normal hormone functions. Both female and male sex hormones, estrogen and androgen respectively, are known to be involved in breast cancer development. In order to measure chemicals that mimic sex hormones, we refined a method using a cell-based assay to simultaneously measure endogenous hormones and exogenous chemical agents in human plasma. The 90 Mexican American female plasma samples were obtained from the San Francisco Bay Area Breast Cancer Study (SFBCS). Results suggest that there is no statistical difference in androgen levels between pre-menopausal and post-menopausal women. In addition, there was also no significant difference between breast cancer cases and the normal control group, possibly due to the small sample size (15 cases). However, we observed a statistically significant difference in estrogen levels between pre-menopausal and post-menopausal women. By using this sensitive and rapid method, we could measure total hormone levels and apply measurements to help identify chemicals that can alter sex hormone functions.

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TECHNICAL ABSTRACT

A Multidisiplinary Field and Laboratory Investigation of the influence of Metals Mixtures on Single Organism (D. Magna) Toxicity and Benthic Community Health in the North Fork of Clear Creek, a Mining Impacted Stream.

In laboratory single-species (D. Magna) aquatic toxicity tests we have found that binary and ternary mixtures of metals (Cd, Cu, Ni, Zn) can display additive, less-than-additive, or greater-than-additive responses. Any given mixture outcome depends on the metals present and the water composition (pH, hardness, alkalinity, dissolved organic carbon). Given that water chemistry is highly variable in acid mine drainage (AMD)-impacted streams, and that multiple metals are customarily present, these observations demonstrate that toxicological models (BLM) will have to quantitatively incorporate the sometimes-competing geochemical interactions among metals. Although our single-species toxicity tests have been enlightening, to understand the complexity of aquatic ecosystem response to AMD, we have performed benthic community studies involving metal exposures in microcosms and at the field site (Central City Superfund site). Species diversity and population density are affected by the AMD sources. We have also characterized the stream sediments, specifically examining iron, aluminum, and manganese oxide precipitates, as they are a repository for potentially toxic metals and also physically impair the suitability of the stream substrate for maintaining benthic habitats. The study is being performed in the anticipation that several of the main AMD sources will be remediated in the next few years. To order to simulate the post-remediation conditions, we have transferred contaminated sediments collected downstream of the AMD sites to an upstream reference site and examined their biological and chemical recovery.

LAY ABSTRACT

A Multidisiplinary Study on the Influence of Biogeochemistry and Metal Bioavailability on the Heath and Potential Recovery of a Stream Affected by Acid Mine Drainage

Abandoned mine sites are a common feature of the landscape of the western US and exposure of sulfide-rich wastes to water and oxygen generates acid-mine drainage (AMD). Introduction of AMD into receiving waters lowers the pH and introduces a mixture of dissolved metals (Fe, Al, Mn, Zn, Cu, Ni, Cd). Stream ecology is highly impaired by the presence of these contaminants in the water column and by the formation of solid precipitates of iron, aluminum, and manganese oxides. Tens of thousands of river miles have been impacted in this way throughout the USA. Stream restoration can be accomplished by treating AMD point sources to remove metals and acidity. However, because the toxic effects of metals and acidity are dependent upon a number of water quality parameters, the extent and speed of aquatic biological recovery is difficult to predict. In this study we take a multidisciplinary approach to performing both laboratory and field experiments to further understand the complexities of metal bioavailability and biogeochemistry in order to make predictions of stream recovery.