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Research Brief 157: SBRP Investigators Find Evidence Suggesting a New Type of Endocrine Disruptor

Release Date: 01/02/2008

Background:

Recent studies have raised concerns regarding the environmental fate of triclocarban (TCC, 3,4,4'-trichlorocarbanilide), an anti-bacterial compound commonly added to a wide range of household and personal care products including bar soaps, detergents, body washes, cleansing lotions and wipes (Halden and Paull 2005). Drs. Jay Chen and Bill Lasley, researchers in the UC Davis SBRP examined TCC's endocrine-disrupting (EDS) effects using a biomarker assay that they had created, since data on TCC's potential impact on reproductive health are scarce and outdated.

Advances:

The research group led by Drs. Chen and Lasley investigated the EDS properties of TCC using their androgen receptor bioassay to determine if the compound interferes with the natural action of endogenous androgens. The cell-based androgen receptor-mediated reporting system uses human embryonic kidney (HEK) cells lacking critical steroid-metabolizing enzymes. The cells were transfected with an androgen receptor (AR) and a plasmid containing a luciferase-reporting gene.

Tested alone at a concentration of 1.0 μM (~0.3 μg/mL), TCC exhibited no significant androgenicity. However, TCC had an amplification effect on strong native androgens such as testosterone (T), and the transcriptional activity was time- and dose-dependent as determined by the fluorescent signal in the androgen receptor assay.

Reports have shown associations between EDS exposures and:

- reduced fecundity and fertility
- abnormal fetal development
- timing of the onset of puberty
- altered steroid hormone biosynthesis
- disruption of ovarian function
- abnormal lactation
- early onset of reproductive senescence
- cancer

To assess the mechanism by which TCC mediates the enhancement of the T-signal, the researchers added the antiandrogen flutamide to the test system. Flutamide blocked the amplification effect of TCC, indicating that the androgen receptor was necessary for TCC's actions. A competitive binding assay was run on TCC with no effect, indicating that TCC does not mimic testosterone and compete for T-binding to the AR.

The researchers' next step was to confirm the biological relevance of the *in vitro* findings by conducting *in vivo* studies. When castrated male rats were exposed to TCC in combination with testosterone propionate (TP), the researchers observed a substantial and significant increase in the weights of all accessory sex organs compared to TCC or TP treatment alone, indicating a synergism between TP and TCC *in vivo*. These data strongly suggest that TCC has a positive androgen receptor modulatory effect in tissues or cells that are androgen targets.

Significance:

These findings suggest that TCC should be categorized as a steroid hormone amplifier or enhancer rather than as a simple agonist or antagonist because of the EDS property of synergism with the native androgen hormone receptor ligand.

The amplification effect of TCC may have an array of widespread subtle physiological alterations in males and females. Hypothetically, amplification of androgens by TCC at the target cell may be associated with idiopathic hyperandrogenism, i.e., despite seemingly "normal" native circulating androgen levels, virilization may occur. It is also conceivable that exposure to TCC may result in defects in development or decreased reproductive function (i.e., sperm quality) in adults. In females, increased androgenic feedback could disrupt the normal female-specific "positive" feedback loop associated with ovulation. Exposure to TCC may also change the balance between estrogen signaling and androgen signaling in breast homeostasis. Depending on the level that hormone signaling pathways are disrupted, *in utero* exposure to TCC could also impair neurogenesis and sexually dimorphic neurobehavioral development. TCC has the potential to amplify synthetic steroidal compounds; as a result, further investigation of the interaction of TCC with oral contraceptives, hormone replacement therapy, synthetic androgens and glucocorticoid therapy is warranted.

Reports indicate that 0.4% of an average 138 mg of TCC (or 0.54 mg) is absorbed after a typical "whole body" shower lather. Therefore, the actual systemic dose of TCC would be ~ 0.1 µg/mL for an adult of 60 kg with 5 L of blood.

Practically, because there is no strong evidence for the improved efficacy of TCC as an anti-bacterial agent in consumer products and as used by consumers, compared to similar products not containing TCC, consumers may want reduce their consumption of TCC-containing products. Likewise, its fate in the environment, especially through the land application biosolids route, and that of another common anti-microbial, triclosan (TCS), should be thoroughly re-examined.

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

Chen, Jiangang, Ki Chang Ahn, Nancy A. Gee, Shirley J. Gee, Bruce D. Hammock, Bill L. Lasley, M.I. Mohamed, A.J. Duleba, and L. Zhao. 2007. Triclocarban enhances testosterone action: A new type of endocrine disruptor? Endocrinology. 149(3):1173-1179. doi:10.1210/en.2007-1057

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Research Brief 158: A Screening Tool to Identify Developmental Neurotoxicants

Release Date: 02/06/2008

Background:

Exposure of pregnant women and children to pesticides and other Superfund chemicals represents a major environmental risk for effects on neurodevelopment. Rapid screening techniques are needed to focus resources on chemicals that pose the greatest potential risk. The process of assessing developmental neurotoxicity is complicated not only by the large number of chemicals and mixtures to be tested, but because the presumed mechanisms and nervous system effects seen in adults may be unrelated to developmental neurotoxicity. For example, it was believed that all organophosphates act similarly — through irreversible inhibition of acetylcholinesterase. Research conducted by Dr. Ted Slotkin at the Duke University SBRP demonstrated that organophosphates' adverse effects on brain development actually involve multiple mechanisms, many of which are unrelated to cholinesterase inhibition (see Research Brief 80).

PC12 Cell Lines:

- Maintain cell division so they can be used to detect adverse effects on the cell cycle, a likely neurotoxic target
- Provide a uniform population in terms of cell types and differentiation state
- Gradually exit the mitotic cycle upon exposure to nerve growth factor (NGF), and begin to differentiate, developing axonal projections, electrical excitability, and the characteristics of two distinct phenotypes, cholinergic and catecholaminergic neurons

Dr. Slotkin's research group has developed an *in vitro* test system using PC12 cells to detect toxicant actions that target cell replication, as well as the events involved in differentiation and the phenotypic emergence of specific neuronal features. This system focuses on aspects of neurodevelopment that lend themselves to rapid screening and that permit ready comparisons of targets involving cell replication, growth, and differentiation.

Advances:

The Slotkin lab used the new test system to compare known developmental neurotoxicants (organophosphate pesticides) with other suspected classes of neurotoxicants including a carbamate pesticide, organochlorine pesticides, heavy metal contaminants, and river sediments from contaminated sites.

With the *in vitro* model, the lab established that the organophosphates chlorpyrifos, diazinon, and parathion are equally damaging to neural cell replication and differentiation at exposure levels below the threshold for significant inhibition of cholinesterase. These effects intensify as the transition to cell differentiation is approached. The researchers also found that the organochlorine pesticide dieldrin and divalent nickel, which act through different originating mechanisms, elicit similar patterns of disrupted neural development. The organophosphates, as well as other agents, interfere with the specification of the cholinergic phenotype; these are the type of neurons that are the most sensitive to damage when assessments are conducted *in vivo*.

The researchers identified replication through early differentiation as the critical stages that are vulnerable to the developmental neurotoxicity of these agents and identified why certain

circuits are targeted. With that information, the lab explored methods of amelioration, depicting that a "cocktail" of protectant drugs can prevent developmental neurotoxicity as a result of organophosphate exposure.

Finally, scientists continue to explore the events in gene transcription that precede the emergence of neurotoxic outcomes as a way of determining if there is a "fingerprint" pattern of gene expression that predicts whether given agents will be developmental neurotoxicants, and what their likely targets and critical periods might be. The lab has successfully defined gene groupings that make these predictions across different classes of compounds with disparate neural targets.

Significance:

These results are important for three reasons:

- They demonstrate that the adverse effects of chlorpyrifos on neurodevelopment are shared by other organophosphates, and also by apparently unrelated Superfund chemicals that can target similar events, such as divalent heavy metals and organochlorines.
- In the case of the organophosphates, these studies point to mechanisms, other than cholinesterase inhibition, as the primary culprits. This suggests that the current use of cholinesterase activity as a biomarker for these adverse effects is inappropriate, necessitating a revised approach to the detection of developmental neurotoxicity.
- This demonstration of an effective cell culture mode to assess the developmental neurotoxicity of various compounds opens the door to rapid screening of suspected environmental neurotoxicants, using a combination of gene transcription "fingerprints" and defined, adverse outcomes.

The information provided by this work has been cited heavily in important documents for pesticide regulation, including the 2006 EPA Inspector General's Report (Report no. 2006-P-00009), "Opportunities to Improve Data Quality and Children's Health Through the Food Quality Protection Act." This research served, and continues to serve, as an endpoint guide for studies of neurodevelopment outcomes in children exposed to pesticides, cited by a number of NIEHS-funded research centers.

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

Slotkin, Theodore A. and Fredric J. Seidler. 2007. Comparative developmental neurotoxicity of organophosphates in vivo: transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. Brain Research. 72:232-274.

Slotkin, Theodore A., E.A. MacKillop, I.T. Ryde, and Fredric J. Seidler. 2007. Ameliorating the developmental neurotoxicity of chlorpyrifos: a mechanisms-based approach in PC12 cells. Environmental Health Perspectives. 115:1306-1313.

Slotkin, Theodore A., E.A. MacKillop, I.T. Ryde, Charlotte A. Tate, and Fredric J. Seidler. 2007. Screening for developmental neurotoxicity using PC12 cells: comparisons of organophosphates with a carbamate, an organochlorine and divalent nickel. Environmental Health Perspectives. 115:93-101.

Research Brief 159: *In utero* and Early Postnatal Exposure to Arsenic May Alter Pulmonary Function

Release Date: 03/05/2008

Background:

Growth and development require the expression of genes and gene products to be temporally and spatially coordinated. text box stating: Research indicates that exposure to high arsenic concentrations in adults can result in lung cancer and/or chronic lung disease. *In utero* and early postnatal exposure to toxicants can affect gene expression, which can alter organ structure and physiological function. University of Arizona (UA) SBRP investigator, Dr. Clark Lantz, and his research team believe that *in utero* and postnatal arsenic exposure via drinking water, at environmentally relevant doses, can result in altered gene expression. These alterations in gene expression may impact lung and blood vessel development, resulting in chronic disease as an adult.

Research indicates that exposure to high arsenic concentrations in adults can result in lung cancer and/or chronic lung disease.

Advances:

Dr. Lantz's team is using a mouse model to explore the developmental effects of arsenic in the lung. To determine if arsenic exposure during critical development could alter pulmonary function, the researchers exposed male and female mice, prior to mating, with up to 100 ppb (parts per billion) arsenic via drinking water. Arsenic exposure continued throughout the pregnancy and after birth. Once the pups were weaned, they were given water with the same level of arsenic for 28 days. The researchers tested the pups' pulmonary function and found that arsenic exposure induced airway hyperreactivity. To determine if this change was permanent, the researchers switched the mouse pups previously exposed to arsenic to control water for 28 days. Pulmonary function tests showed no reversal in airway hyperreactivity, suggesting that alterations induced by *in utero* and early postnatal exposures to arsenic are not reversible with the removal of arsenic.

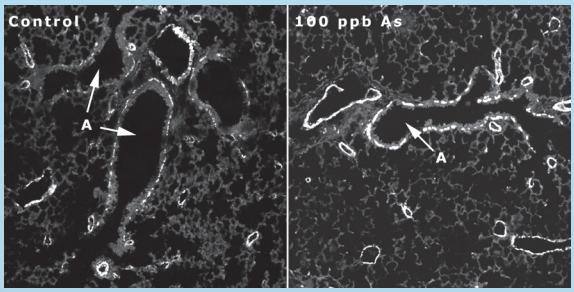

Figure 1: Immunostaining for smooth muscle actin in control and 100 ppb arsenic exposed animals. Tissues were collected from mice exposed *in utero* and/or up to 28 days postnatally. There is an increase in the level of smooth muscle actin staining around airways (A) in the arsenic exposed animals.

Adult mice exposed to arsenic via drinking water for four weeks did not show the same arsenic-induced increase in airway reactivity as seen in mice exposed during *in utero* and early postnatal development.

To determine how arsenic can cause these functional alterations, Dr. Lantz's team is examining anatomical changes in the airways and in tissues surrounding the airways. In humans, airway hyperreactivity is associated with increased levels of connective tissue and smooth muscle around the airways; the lab is currently looking at these parameters. Dr. Lantz's data indicate that the expression levels of some extracellular matrix genes are altered by arsenic, and there is an increase in the amount of smooth muscle that surrounds the airways. The researchers are applying quantitative anatomical analyses to verify these changes.

Significance:

These findings may impact future public health policies by providing an intervention strategy to reduce adverse human health outcomes associated with arsenic exposure. It appears that the timing of exposure is important for inducing the alterations. For instance, *in utero* and early postnatal exposure can cause alterations in pulmonary function, while arsenic exposure, exclusively during the adult years, does not. Once exposure is established, alterations in pulmonary function cannot be reversed simply by removing the arsenic, which suggests that the ***timing of intervention strategies is important to reduce adverse effects.***

These data also suggest that children exposed to arsenic during periods of critical development would have an increased risk of developing lower respiratory infections (LRI), which are a significant cause of infant morbidity and mortality in developing countries. Identifying environmental agents that may lead to increased LRIs, in addition to reducing exposure of pregnant mothers and young children to environmental agents, can, potentially, improve health in third world countries.

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Research Brief 160: High-Performance Carbon-Based Mercury Sorbents

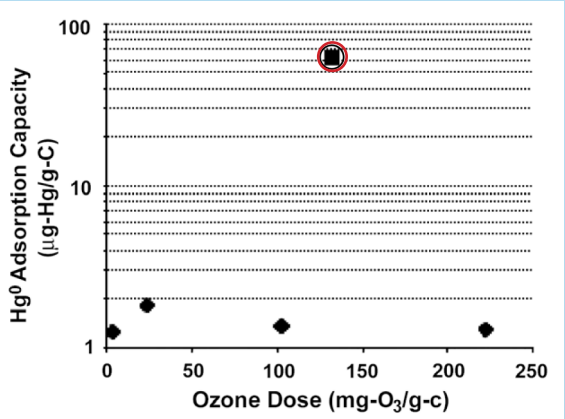
Release Date: 04/02/2008

Background:

Mercury (Hg) is typically oxidized or methylated in the environment before reaching human receptors. However, mercury is often in the elemental state at the point of release, where concentrations are highest and the conditions for capture are most favorable. Dr. Robert Hurt at the Brown University SBRP is working to improve the efficiency of elemental mercury capture by chemical modification of carbon sorbent surfaces. The results of this work may be applicable to sorbent-based capture of mercury for spill scenarios, remediation technologies, respirator cartridges, incineration systems, and the largest application: coal-fired power stations.

Advances:

Using carbon black and mesoporous carbon (matrix with pores 2-50 nm in diameter) as model systems, Dr. Hurt's research group studied the effects of modifying carbon surfaces by oxidation. Early experiments indicated no significant enhancement of mercury capture efficiency by oxidation with ozone, hydrogen peroxide or air. However, as shown by the square symbol in the figure, a single experiment gave a very different result: a nearly 48 fold capacity increase. Rather than discard this outlier, Dr. Hurt's group investigated and found that the data point was from a sample treated at high ozone concentration and immediately tested for mercury adsorption capacity. The researchers conducted additional experiments with freshly ozone-treated carbon materials at a range of ozone doses. They confirmed that a dramatic capacity increase occurs when the adsorption takes place within 30 minutes of ozonolysis. For example, capacity increases as high as 134 fold were observed with carbon black samples. Interestingly, oxidation of carbon black with hydrogen peroxide or air did not lead to a significant capacity increase.



Effect of ozonolysis degree on total Hg adsorption capacity.

To better understand this unique, transient surface activation, Dr. Hurt's research group conducted variety of aging studies on ozone-treated carbon blacks. They observed decreases in mercury adsorption capacity within 24 hours when freshly treated carbon black was exposed to room air or water vapor. Flushing freshly treated carbon black with nitrogen at a high temperature (120°C) completely destroyed the capacity enhancement in less than four hours.

Dr. Hurt hypothesized that ozone treatment of carbon results in the formation of surface functional groups with oxidizing ability. These functional groups might further oxidize the carbon substrate through decomposition, or oxidize adsorbate species such as mercury. To learn about the chemical structure of the functional groups, the researchers conducted FTIR spectroscopic analyses of untreated, ozonated, and aged/ozonated carbon black samples. These studies suggest that epoxides and (epoxide-containing) secondary ozonides are the most likely candidates for the surface functional groups.

Significance:

Mercury contamination is both a local and a global problem. Understanding mercury/sorbent chemistry is important to guide the manufacture, selection and use of simple sorbent systems. Dr. Hurt's findings suggest potential for in situ carbon ozonolysis to create fresh, super-active sorbents.

The group is now expanding this work to include new nano-sorbents for capture of Hg vapor released from broken compact fluorescent lamps.

Because carbon is a nearly universal environmental sorbent, used with both heavy metals and chlorinated organics (e.g., dioxins, furans, TCE), such fundamental research on optimal sorbent formulation and surface chemistry could have wide applicability.

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

Manchester, S., X. Wang, Indrek Kulaots, Yu-Mine Gao, and Robert H. Hurt. 2008. High capacity mercury adsoprtion on freshly ozone-treated carbon surfaces. Carbon. 46(3):518-524. doi:10.1016/j.carbon.2007.12.019

Research Brief 161: PCB77 Promotes Obesity-associated Atherosclerosis

Release Date: 05/07/2008

Background:

Polychlorinated biphenyls (PCBs) are industrial chemicals that bioaccumulate in the ecosystem. PCBs are highly lipophilic and accumulate markedly in fatty tissues, so obesity could increase the body burden of PCBs. Although adipocytes (fat cells) are likely to be frequently exposed to PCBs, the specific effects of PCBs on adipocyte function have not been defined. Moreover, the relationship between increased PCB body burden with obesity and the development of obesity-associated diseases is unknown.

Dr. Lisa Cassis (University of Kentucky SBRP) and her research team are investigating the impacts of PCB exposures on the development of obesity and the mechanisms that link obesity to cardiovascular disease. Studying PCB77, a "dioxin-like" coplanar arylhydrocarbon receptor (AhR) ligand, they are working to:

- Define the concentration-dependent effects of PCB77 on the development of obesity.
- Examine the effects of PCB77 on adipocyte production of pro- and anti-inflammatory factors.
- Investigate the effects of adipocyte-sequestered PCB77 on alterations in serum lipids and development of atherosclerosis.

Advances:

Dr. Cassis' team conducted both *in vitro* and *in vivo* studies in rodents. In the first series of experiments, they examined the concentration-dependent impacts of exposures to PCB77 or dioxin (tetrachlorodibenzo-p-dioxin, TCDD) on rates of preadipocyte differentiation to mature adipocytes. They found that low concentrations of PCB77 and TCDD ***increase*** adipocyte differentiation, resulting in an increase in body weight, adipose mass and adipocyte area. Higher concentrations ***inhibit*** adipocyte differentiation.

Effects of PCB77 were abolished by the AhR antagonist, α-naphtoflavone (α-NF). The research team also showed that PCB153, which has a low affinity for interaction with AhR, did not impact adipocyte differentiation. These findings suggest that PCB77 and TCDD effects on adipocyte differentiation and body weight are AhR-mediated.

Dr. Bernhard Hennig, also an SBRP-funded researcher at UK, previously demonstrated that PCB77 promotes inflammation in endothelial cells.

Obesity is associated with an elevation in the systemic concentrations of adipokines (cell-to-cell signaling proteins) that have been linked to obesity-related diseases including coronary artery disease. Dr. Cassis conducted a study to determine if PCB77 impacts adipokine secretion from adipocytes. Such an effect would influence not only obesity, but also obesity-associated atherosclerosis. The researchers found that PCB77 promotes the expression and secretion of a variety of proinflammatory adipokines, and decreases the expression of an anti-inflammatory adipokine (adiponectin). To our knowledge, this is the first report demonstrating that PCBs can promote the production of these factors from adipocytes.

Dr. Cassis' research also revealed that exposure to low doses of PCB77 results in a marked increase in serum cholesterol concentrations, with predominant increases in very-low-density lipoprotein (VLDL) or "bad" cholesterol. In addition, the elevations in VLDL-cholesterol by PCB77 were associated with increased atherosclerosis. To the researcher's knowledge, this is the first study that has directly examined the effects of PCB exposure on experimental atherosclerosis.

Significance:

Though production of PCBs was banned in 1977 in the United States, PCBs are persistent, and biomagnified, in the environment. On-going sources of potential human exposures include fish consumption, soil, groundwater and drinking water. At the same time, the prevalence of obesity in both adults and children continues to increase at an alarming rate.

Dr. Cassis' groundbreaking research reveals that even low level exposure PCBs may contribute to the development of obesity and obesity-associated atherosclerosis. This discovery of the inter-relationships between PCBs, obesity, and obesity-related diseases could inform public health policy and practice.

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

Arsenescu, V., R.I. Arsenescu, V. King, Hollie Swanson, and Lisa A. Cassis. 2008. Polychlorinated Biphenyl-77 Induces Adipocyte Differentiation and Proinflammatory Adipokines and Promotes Obesity and Atherosclerosis. Environmental Health Perspectives. 116:761–768. doi:10.1289/ehp.10554

Research Brief 162: Toxicogenomics Studies Focus on Largemouth Bass

Release Date: 06/04/2008

Background:

The goal of ecotoxicology is to measure and predict the impact of pollutants on populations, communities, and ecosystems. New analytical tools, such as polymerase chain reaction (PCR) and microarrays, allow for gene expression analysis studies that identify biochemical pathways altered by exposure to environmental pollutants. This information can reveal modes of action of toxic chemicals and shed light on impacts of exposures to mixtures of pollutants. However, for such "omics" technologies to gain acceptance in ecotoxicology and application in risk assessment, scientists will need to define how biomarkers identified at the molecular level can be used to predict adverse effects in individuals, populations, and various species.

Cytochrome (CYP) enzymes catalyze the oxidative metabolism of xenobiotic and endogenous compounds, yielding less toxic forms that are easier for the body excrete.

In fish, members of the CYP2 and CYP3 enzyme families play a major role in steriod metabolism.

Although gene expression analyses have been used extensively in mammalian toxicology, applications have been limited in aquatic systems, primarily because the genome sequencing for relevant species is still in its early stage. With funding from the Superfund Basic Research Program (SBRP), Drs. Nancy Denslow and David Barber at the University of Florida are designing microarrays ("gene chips") for studies to examine the impacts of organochlorine pesticide (OCP) contamination on fish.

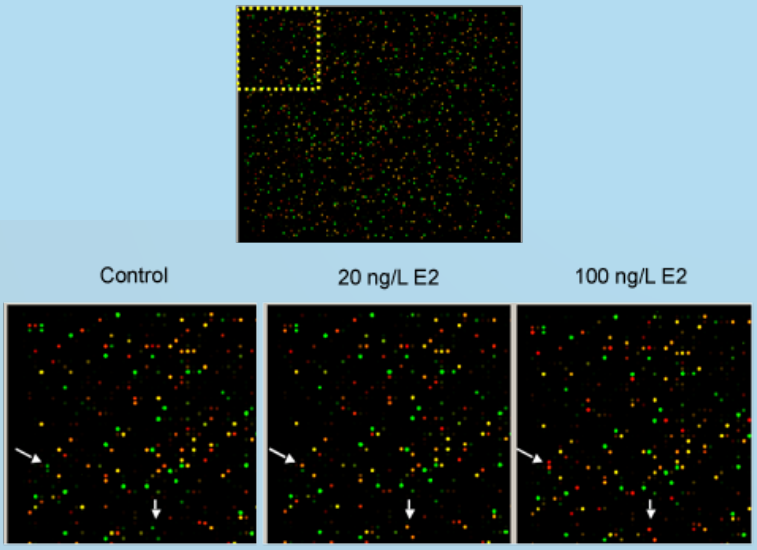
Advances:

Drs. Denslow and Barber study poorly-reproducing, non-flourishing populations of largemouth bass (Micropterus salmoides– LMB) in Central Florida lakes. These ecosystems are contaminated with high levels of OCPs, including p,p'-DDE (a DDT residual) and dieldrin . In initial studies, Drs. Denslow and Barber showed that plasma steroid levels were abnormal in fish from contaminated sites, which demonstrated that they could reproduce the altered profiles in fish fed diets containing p,p'-DDE or dieldrin (Garcia-Reyero et al., 2007). Dietary exposure to these compounds affected gene expression related to steroid homeostasis (steroid biosynthesis and metabolism), and hormone receptor signaling in both the liver and gonad of treated animals. These results suggest that OCPs have complex effects on the endocrine system, likely involving direct effects on steroid receptors as well as altered signaling by endogenous hormones.

Among the genes tested were steroid hormone receptors, genes involved in steroidogenesis (StAR and aromatase), and genes involved in metabolism, including two isoforms of CYP3 (CYP3A68, CYP3A69) and CYP2 (CYP2N14, and CYP2P11). The team determined that expression of the CYP enzymes changes during the reproductive cycle of LMB and differs between sexes (Barber et al., 2007).

To delve further into the mechanisms underlying the effects of OCPs, a custom microarray for LMB was designed by sequencing a normalized cDNA library using the 454 Life Sciences GS-20 pyrosequencer (Garcia-Reyero et al., 2008). This novel approach netted a total of 31,391 unique

sequences from which 16,350 annotated individual transcripts were selected for the design of the microarray. The microarray was validated by examining the transcriptomic response in livers and gonads of male LMBs exposed to 17ß-estradiol. Based on pathway analyses, changes in the liver appeared to be primarily due to ER signalling and metabolism, whereas changes in the gonad primarily involved steroidogenesis, apoptosis, and metabolism. The quantum leap in speed and cost of high-quality sequence data generated by the pyrosequencing process depict a huge improvement in the effort of developing high-quality microarrays for ecologically relevant species.



Example of an oligonucleotide microarray developed for a non model fish species. The square on the top right corner is expanded below to show the results of exposure of male fish to a vehicle control, 20 ng/L E2 or 100 ng/L E2. A reference design was used where the reference sample made of equal amounts of total RNA from males and females (liver, gonad and brain) was labeled with the green fluor and the samples (liver from control, 20 ng/L E2 and 100 ng/L E2 exposures) were labeled with the red fluor. The arrows point to two spots that go from green (expressed more in the reference sample) to orange and then red (increased expression in the exposed samples). The spots correspond to Vtg mRNA.

The array is used to examine transcriptional changes evoked by exposure to OCPs in both laboratory- and field-exposed fish. In one study, the team investigated the effects of dieldrin on the neuroendocrine system, primarily targeting the hypothalamus in the LMB brain. Dieldrin is a GABAergic antagonist, blocking GABAergic neurotransmission, which is an important regulator of neuroendocrine signaling. Over 100 genes in the hypothalamus of LMB were significantly altered by exposure to dieldrin, including genes that 1) have previously been implicated in Parkinson's disease, 2) are indicative of brain damage and apoptosis, and 3) are involved in hormone and cell signaling.

Significance:

These results demonstrate that long term exposure of LMB to OCPs, during their reproductive phase, produces complex compound- and gender-specific effects that involve all parts of the fish endocrine system from the hypothalamus to the gonad and liver. This work also demonstrates the potential to rapidly create the tools necessary to assess large scale transcriptional responses in non-model species, paving the way for expanded impact of toxicogenomics in ecotoxicology. Future studies by the group will utilize compounds with known mechanisms of action to characterize the mechanisms of individual OCPs, as well as mixtures.

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

Denslow, N.D. 2008. Construction of a robust microarray from a non-model species (largemouth bass) using pyrosequencing technology. Journal of Fish Biology. 72(9):2354-2376(23). doi: 10.1111/j.1095-8649.2008.01904.x

Barber, David S., N.D. Denslow, and Natalia Garcia-Reyero. 2007. Fish 'n' chips: the use of microarrays for aquatic toxicology. Molecular BioSystems. 3:172-177.

Barber, David S., A.J. McNally, Natalia Garcia-Reyero, and Nancy D. Denslow. 2007. Exposure to p,p'-DDE or dieldrin during the reproductive season alters hepatic CYP expression in largemouth bass (*Micropterus salmoides*). Aquatic Toxicology. 81(1):27-35.

Research Brief 163: The Role of Soil Bacteria in Phytostabilization of Mine Tailings

Release Date: 07/02/2008

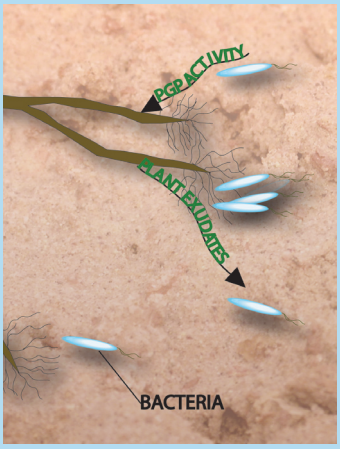
Background:

Tailings piles at abandoned mines contain toxic levels of metals, contain no organic matter, are low in nutrients, and are often quite acidic; as a result, the piles generally have minimal vegetation or are completely barren, making them vulnerable to wind and water erosion. The dispersion of tailings spreads particles across large geographic areas, contaminating ground and surface water, and exposing humans and animals to significant levels of fine particulate matter (PM2.5).

In the United States there are ~550,000 abandoned mine sites, which have generated 45 billion tons of mine waste.

Removal and in situ treatment are not economically feasible at most mining sites. Chemical and physical stabilization technologies have been used, but are expensive and may not be permanent solutions. Phytostabilization, establishing a vegetative cap for the long-term stabilization and containment of tailings, is a promising remediation strategy that could reduce remediation costs as well as re-establish healthy, stable ecosystems. Dr. Raina Maier at the University of Arizona SBRP leads a team of researchers working to optimize phytostabilization strategies for mine tailings sites in semiarid locations. Her group conducts extensive greenhouse and field studies and has identified nine native plants suitable for phytostabilization projects. They are working to minimize the amounts of organic matter (compost) and water needed to establish and maintain the vegetation.

Advances:



Buchloe dactyloides (buffalo grass) in non-composted Klondyke tailings demonstrating enhanced growth when inoculated with isolated PGPB's.

Dr. Maier's group works on-site and with samples collected from the Klondyke mill site in Aravaipa Valley, Arizona. This state Superfund site contains ~70,000m3 of acidic tailings with elevated levels of arsenic, cadmium, lead, and zinc. The researchers' current work focuses on the role of soil bacteria in establishing healthy plant communities. Diverse heterotrophic microbial communities can support plant growth via a variety of mechanisms, including suppressing plant pathogens; producing growth hormones; and increasing availability and uptake of nutrients.

One strategy being explored to enhance revegetation is inoculation of seeds with plant growth-promoting bacteria (PGPB). To identify bacteria with potential of plant growth-promoting (PGP) activity, the researchers began with 131 bacterial isolates and identified 20 that are metal tolerant and able to grow in acidic conditions. The researchers then evaluated the 20 isolates for the ability to enhance the growth of two native plants (quailbush and buffalo grass), while reducing the amount of compost necessary to establish the plants. Previous work by Dr. Maier's group demonstrated that 15% (W/W) compost addition restored growth to levels comparable to that observed in an off-site soil control, however, treatments of 0% and 10% did not.

The researchers evaluated bacterial isolates for the ability to enhance plant growth at the 0% and 10% suboptimal compost treatment levels by measuring plant survival and plant biomass. They found that:

- In 0% amended treatments, some of the isolates increased plant survival, but did not enhance plant biomass. The researchers believe these isolates be used as part of a PGPB mixture to enhance overall survival and growth.
- Some of the isolates that yielded the greatest plant biomass increase in the 10% compost treatment were not effective in the 0% compost treatment.
- The impact of PGPB was plant specific: buffalo grass responded more strongly to the PGPB at the 0% compost treatment, whereas quailbush responded more strongly in the 10% compost treatment.
- PGP activity of some isolates changed over time, suggesting that environmental factors (e.g., pH, metal toxicity) may impact long term survival of bacteria in the zone surrounding the roots of plants (rhizosphere).

In a separate study, Dr. Maier's group used phylogenetic analysis to compare microbial communities from moderately acidic and extremely acidic areas of the Klondyke site to an off-site control area. The microbial communities in the control area samples were significantly more diverse than those from the mine site, and contained more types of heterotrophic bacteria. The samples from the moderately and extremely acidic areas were not only less diverse, but contained iron- and sulfur-oxidizing bacteria not found in the control sample. Dr. Maier believes that the presence or absence of heterotrophic bacteria could serve as a bioindicator of the degree of site disturbance. Such a profile could be usedto assess the potential for plant establishment at a site.

Significance:

Dr. Maier's research demonstrates the important role of bacteria in the phytostabilization of mine tailings. Specific populations of endemic bacteria in tailings should be assessed and, perhaps, monitored. Introduced populations of bacteria that serve as PGPB can successfully enhance the growth of native plants in mine tailings from arid environments and partiallyreplace the requirement for compost amendment. This work highlights the importance of identifying PGPB that not only provide plants with key PGP mechanisms, but that can persist in the tailings rhizosphere.

Klondyke State Superfund Site (adjacent to the Aravaipa Creek near Klondyke, Arizona) has remained unvegetated for over 50 years. Mine tailings are composed of fine to coarse sand sized particles with the consistency of flour.

Dr. Maier emphasizes the need for monitoring the long-term success of phytostabilization projects. Plants must not only survive and grow, but must self-propagate to colonize the area with no additional inputs. In addition, it will be critical to monitor changes in the rhizosphere as evolving microbial communities and weathering and decomposition of organic materials may result in conditions that alter the speciation, bioavailability, and phase distribution of metal contaminants.

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

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Research Brief 164: New Nanomaterials to Capture Mercury Vapor Released from Compact Fluorescent Lamps

Release Date: 08/06/2008

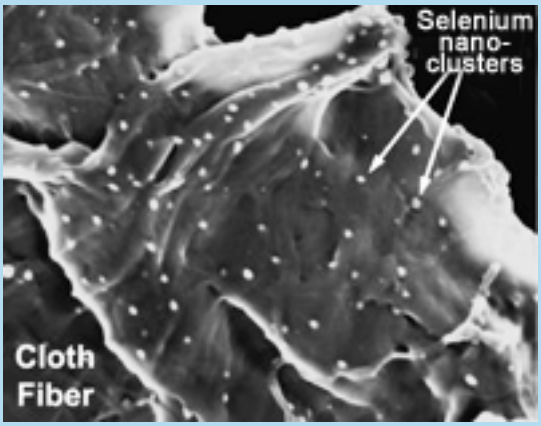
Background:

The switch from incandescent light bulbs to energy-efficient compact fluorescent lamps (CFL) represents a potential safety issue as CFLs contain 3 to 5 mg of mercury (Hg). Mercury is a neurotoxin of special concern for infants, children, and pregnant women. Dr. Robert Hurt, Director of the Institute for Molecular and Nanoscale Innovation at Brown University, and leader of an SBRP-funded research project, directs a team of scientists studying mercury vapor release from broken and spent CFLs.

Dr. Hurt's team, which includes engineering student Natalie Johnson, and graduate student Love Sarin, is working to address direct and indirect exposure issues related to the management of Hg from CFLs. Direct exposures to Hg can occur when lamps are broken during shipping, retail sales, consumer use, or recycling. Broken CFLs release Hg as a volatile vapor; 80% of inhaled Hg is physiologically absorbed. Indirect exposures can occur as the result of disposal. Mercury that leaches from a landfill or escapes in landfill gases can enter food webs, bioaccumulate, and eventually reach human food sources. Dr. Hurt's research focus is on the development of methods for Hg vapor capture and stabilization at ambient temperatures.

Advances:

With the ultimate goal of identifying new high-efficiency sorbents to capture Hg from broken CFLs and/or for use in recycling efforts, Dr. Hurt's team began by characterizing the timing and extent of Hg released from broken CFLs. They found that initial Hg release is quite rapid (yielding concentrations in air near the bulbs of 200-800 µg/m3 during the first hour) and continues at significant levels for several days. In about four days, broken CFLs release ~30% of total Hg (1mg), leaving much of the Hg in the bulb debris to evaporate slowly.



This electron microscope image shows the internal structure of the active sorbent lining. *(Credit: Division of Engineering, Brown University)*

Next, the researchers evaluated candidate Hg sorbents. Based on the knowledge that Hg vapor capture on solids is largely dependent on surface area, they focused their attention on high-area, nanoscale formulations of common Hg sorbents. They determined the adsorption capacity of 28 sorbents used for Hg capture including sulfur-containing materials; metals and metal oxides; metal sulfides; carbon; selenium (Se); and two commercially available products. The adsorption capacities ranged from 0.005 to 188,000 µg/g. To identify sorbents most suitable for use for clean up or recycling applications, theresearchers calculated the amount of each sorbent required to capture 1 mg of Hg vapor at room temperature. They found that for some of the more common Hg sorbents (powdered sulfur or zinc), enormous amounts of material (>10 kg) were required; >10 g was required for most sorbents tested. The most effective sorbent was uncoated nanoselenium: <10 mg

captured more than 99% of the Hg vapor over a 24-hour period. In the body, Se sequesters Hg into insoluble and metabolically inactive mercury selenides. In the environment, the stable sequestration of Hg by Se may reduce its mobility, bioavailability, and ecotoxicity.

Dr. Hurt's group has engineered prototype sorbent-containing packaging and disposal bags to allow safe handling of CFLs. Their design inserts a 3-layered nanoselenium lining in retail packaging for CFLs. If a CFL is broken, the packaging can be placed over the area to absorbthe Hg vapor from the spill. The lining can also capture the Hg if the bulb is broken in the box. The researchers have also created a lining for plastic bags to soak up the Hg left over from the CFL shards that are thrown away. The Hg-absorbent packaging and the lined plastic bags can be safely discarded or recycled.

Significance:

Fewer than 2% of CFLs are currently recycled though this fraction may increase in the future. Although biologically significant direct exposure to Hg is unlikely to occur from breakage of a single CFL, there may be some risk to unborn or small children upon continual habitation at break sites, especially involving multiple bulbs that are not properly cleaned up, or in shipping or at recycling centers where large numbers of bulbs are collected in confined spaces.

Dr. Hurt's research has generated an inexpensive, effective solution that could address many safety and public health concerns associated with CFLs. Brown University has applied for federal patents covering the mercury-absorption packaging and the absorbent material and plans to begin discussions with companies on manufacturing the new technology.

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

Hurt, Robert H., S. Manchester, Yu-Mine Gao, Indrek Kulaots, N.C. Johnson, and L. Sarin. 2008. Mercury Vapor Release from Broken Compact Fluorescent Lamps and *In Situ* Capture by New Nanomaterial Sorbents. Environmental Science and Technology. 42 (15), 5772–5778, 2008. doi: 10.1021/es8004392

Research Brief 165: PON1 as a Potential Treatment for Organophosphate Poisoning

Release Date: 09/03/2008

Background:

The paraoxonase enzymes (PON1, PON2, and PON3) play important roles in gene-environment interactions, drug metabolism, and susceptibility to vascular and infectious disease. Since 2000, SBRP-funded researchers at the University of Washington (http://tools.niehs.nih.gov/sbrp/programs/Program_detail.cfm?Project_ID=P42ES4696/) (UW) have conducted a series of investigations to determine: 1) the role of PON1 in modulating the toxicity of mixtures of organophosphate (OP) compounds, and 2) the direct impacts of PON1 on individuals' susceptibility to pesticide poisoning and cardiovascular diseases.

> The World Health Organization estimates that ~3 million pesticide poisonings occur each year worldwide, resulting in 220,000 deaths.

Drs. Lucio Costa and Clement Furlong began with basic biomolecular studies to increase our understanding of PON1 function and modulation. They developed a strategy to characterize both the PON1 genotype (polymorphisms at position 192 in the coding sequence) and phenotype (plasma levels of PON1) of an individual, allowing them to establish an accurate measure of total PON1 activity. Drs. Costa and Furlong have used this "PON1 Status" biomarker in studies to examine the association of PON1 and an individual's susceptibility to environmentally-induced diseases, including Parkinson's Disease and atherosclerosis.

Advances:

Because PON1 can inactivate a wide range of OPs, including metabolites of the pesticides chlorpyrifos, diazionon and parathion, and the nerve agents sarin, soman, and VX, the researchers proposed that PON1 might be an excellent candidate for treatment of OP exposures. They demonstrated PON1's therapeutic potential by injecting PON1 into control rats and mice and then into PON1 knockout mice (i.e., animals completely devoid ofplasma PON1 activity) both before and after OP exposure. The injected PON1 increased or restored PON1 levels in the knockout mice and conferred resistance to the highly toxic metabolites of chlorpyrifos and diazionon exposure, but not to parathion exposure. This work showed the importance of PON1 Status by revealing that the two different PON1 192 variants have different catalytic efficiencies, which, together with plasma PON1 level, determine whether PON1 can protect against a specific OP exposure.

In work published on-line (August 18, 2008) in the Proceedings of the National Academy of Sciences of the United States of America (http://www.pnas.org/), Dr. Furlong presents the results of work aimed at purifying native and engineered PON1 (rHuPON1) from E. coli, and characterizing the potential of PON1 as a therapeutic agent to treat OP exposures.

The UW team injected the PON1 variants into PON1 knockout mice and found that:

- rHuPON1 was not toxic.
- rHuPON1 had a half-life comparable to the native PON1, with plasma levels peaking ~8 hours after injection and persisting for 48 hours.
- rHuPON1 protected the PON1-deficient mice from OP doses up to three times the median lethal dose value when injected before or after exposure.

While conducting this research, the UW team also resolved a procedural challenge that had hampered other laboratory groups who were unable to express and purify active rHuPON1 from bacterial cells. They learned that successful expression of active rHuPON1 depends on the media used and on the temperature for growth. The E. coli system did not produce active rHuPON1 when grown at temperatures of 37°C or above.

Significance:

This work is the first successful expression and purification of enzymatically active, native and engineered recombinant human PON1 from E. coli. Moreover, these experiments clearly demonstrate the therapeutic potential of E. coli-expressed engineered rHuPON1. Dr. Furlong believes that variants with even higher catalytic efficiencies should be attainable and could prove useful for treating OP pesticide and nerve agent exposures.

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

Furlong, Clement E., Rebecca J. Richter, T.B. Cole, S.S. Park, S.M. Suzuki, and R.C. Stevens. 2008. Engineered recombinant human paraoxonase 1 (rHuPON1) purified from Escherichia coli protects against organophosphate poisoning. Proceedings of the National Academy of Sciences of the United States of America (PNAS).105(3):12780-12784.
doi: 10.1073/pnas.0805865105

Research Brief 166: Understanding Dermal Exposure – a Critical Step in Exposure Assessment

Release Date: 10/01/2008

Background:

The skin is a major target for chemical exposure via contact with aerosols, vapors, liquids, contaminated soils, or other contaminated surfaces. The importance of dermal exposure has become more evident as inhalation exposure decreases as a result of improved preventative/personal protection measures. The extent and significance of dermal exposure remain largely unknown, primarily because there is a lack of standard instruments and methods for measuring chemical-specific dermal exposure. Methods are not only needed to quantify exposure, but to also evaluate the relationship of dermal exposure to systemic exposure.

A volunteer patiently sits as he is tested for dermal exposure via the tape-stripping method.

Dr. Leena Nylander-French, University of North Carolina at Chapel Hill SBRP, leads a team of researchers working to develop quantitative tools to collect critical information for exposure and risk assessment.

Advances:

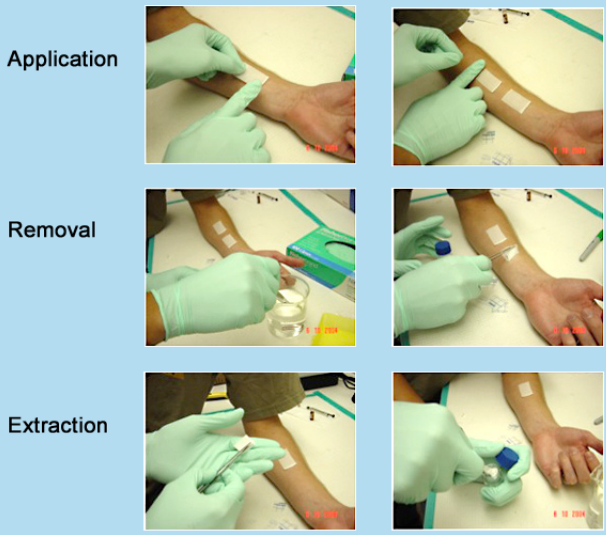
Dr. Nylander-French's research group has conducted an extensive series of studies focused on dermal exposure to polycyclic aromatic hydrocarbons (PAHs). To begin, Dr. Nylander-French developed and validated a non-invasive sampling technique, coupled with analytical methods (GC/MS; ELISA), to quantify dermal exposure to naphthalene as a chemical marker of exposure to fuels. The tape-stripping method involves applying a strip of adhesive tape to an area of skin following exposure, then removing the tape along with the contaminant and several micrometers of the outermost layer of skin. Repeated stripping of the skin can indicate penetration of the substance (depth-profile) and the spatial/temporal variations of different hazardous compounds in the skin.

Dr. Nylander-French used these methods to conduct laboratory and field studies of exposure to jet fuel (JP-8; using naphthalene as a marker) to evaluate the contribution of dermal exposure to total exposure. Her research group examined the correlations between dermal exposure to naphthalene measured with tape-strip samples of exposed skin and breathing zone, exhaled breath, and urine concentrations of napthol metabolites. The data show that the tape-stripping method is an effective technique for measuring dermal exposure, and that:

- The skin is an important route for PAH exposure as JP-8 components penetrated into the skin rapidly with minimum evaporation from the skin surface.
- Dermal exposure to JP-8 contributed significantly to total body burden.
- Dermal exposure correlated highly with (1) the work situation (work inside the fuel tank) and the exposure duration; (2) protection control (use of booties); and (3) a potential health risk (skin irritation).

Dr. Nylander-French's group then developed an enzyme-linked immunosorbent assay (ELISA) to detect and quantify protein adducts of metabolites of naphthalene in the skin of exposed individuals. This work, the first to report of adducts of PAHs detected in exposed intact human skin, provides biomarkers of exposure allowing researchers to: distinguish acute and chronic exposures; investigate the importance of dermal penetration, metabolism, and adduction to keratin; and to more accurately predict the contribution of dermal exposure to systemic dose for use in exposure and risk assessment models.

Dr. Nylander-French has also contributed to the development of mathematical models to describe the uptake of PAHs into the skin. The epidermis is a complex organ with many metabolic functions. Many models of dermal absorption and penetration assume that diffusion is the dominant mechanism of transport and are based on the properties of the contaminant (molecular weight, solubility, reactivity). Dr. Nylander-French's group developed a physiologically-based pharmacokinetic (PBPK) model of exposure to JP-8 that incorporates inhalation and dermal uptake and they have validated the model with experimental human data.



The tape-stripping method involves the application of a strip of adhesive tape to an area of skin following exposure. The tape is removed with the contaminant and several micrometers of the outermost layer of skin.

Dr. Nylander-French has also developed a mathematical model to quantify the penetration of chemicals deposited on the skin surface into its deeper layers. This diffusion-based model assumes that the stratum corneum (outermost skin layer) is the rate-limiting barrier to the penetration of lipophilic compounds. The research group used the model to predict the variation in dermal absorption and penetration of three aromatic and three aliphatic hydrocarbons in JP-8. They found that the model outputs agreed with values measured from *in vitro* skin systems and biomonitoring studies.

In on-going work, Dr. Nylander-French is investigating the capacity of the skin to metabolize PAHs. Because metabolites (not parent PAHs) cause toxic effects, her group is investigating patterns of gene and protein expression in the epidermis that are critical to PAH-exposure response. Quantification of differences in biomarker response and sources of variance in response to exposure levels could predict individual differences and, thus, individual susceptibility to toxicity and associated disease.

Significance:

Dr. Nylander-French has demonstrated that the tape-stripping method is an effective technique for measuring dermal exposure. This body of work makes significant contributions by providing much-needed tools to investigate the significance and role of dermal exposure to hazardous chemicals. The ability to quantify dermal exposure and evaluate its contribution to total exposure and internal dose is critical to the development of exposure and risk assessment models.

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

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Research Brief 167: SBRP Researcher Finds Previously Unidentified PCB in Urban Airshed

Release Date: 11/05/2008

Background:

Polychlorinated biphenyls (PCBs) are a family of low-volatility compounds manufactured extensively across the United States and Europe between the 1920s and the 1970s. Due to their stabilizing and insulation properties, PCBs were commonly used as coolants for capacitors and transformers, as insulation in electrical wiring, and to a lesser extent as plasticizers in paint, caulk, and other sealants. Though they were banned in 1977 over concerns of demonstrated environmental stability and bioaccumulation as well as potential carcinogenicity, PCBs continue to be volatilized into the environment from old disposal sites and through the breakdown of manufactured materials which contain them. As a part of the University of Iowa SBRP, Dr. Keri Hornbuckle is working to determine sources of PCBs observed in ambient air in urban Chicago using a novel sampling method developed for this project. The goal of this research is to identify "hotspots" of PCB volatilization in the urban airshed, characterize the spatial extent of contamination in the city, and estimate the strength of the emission sources.

Advances:

Field sampling in urban areas has historically been a problem due to the logistics of setting up sampling equipment, maintaining the system, and transporting samples for analysis once they are complete. If the logistical difficulties can be surmounted, field sampling at multiple sites is often prohibitively expensive. In collaboration with Dean Macken, Director of the University of Iowa's Engineering Design and Prototyping Center, Dr. Hornbuckle developed a high-volume (Hi-Vol) sampling system that can be mounted on the back of medical vans operated by the Mobile C.A.R.E. Foundation of Chicago. These vans are used for on-site care at local elementary schools for children with asthma and other respiratory diseases. Mobile C.A.R.E. lent assistance to Dr. Hornbuckle in sample collection for this study.

Rodger Peck (Mobile C.A.R.E.), Keri Hornbuckle, and Dean Macken

The Hi-Vol system consisted of a quartz fiber filter for particle sampling in series with an XAD-2 resin cartridge to capture PCBs in the gas phase. Two vehicles were used, which allowed sampling to occur at two different sites on the same day. When a van arrived at a care location, the Hi-Vol system was set up by the driver; samples were collected for the six to eight hours in which the van was parked at the school. Temperature and humidity data were also collected.

The researchers analyzed samples with GC/MS/MS, using a variant of the current EPA method (EPA 1668A). They chose to use a SP-Octyl capillary column instead of a nonselective column and multiple reaction monitoring (MRM) instead of electron capture detection (EC) mass spectrometry. These changes permitted the researchers to resolve 209 congeners into 169 peaks and also allowed for the positive identification of PCB11 in their assay. PCB11 is a known byproduct of 3-3'-dichlorobendzidine and has been otherwise identified in waste from paint and pigment industries. Very little study of PCB11 has been undertaken and its toxicity is currently

unknown. Dr. Hornbuckle found PCB11 in 91% of the samples taken; it contributed to, on average, 5% of total PCB mass. This concentration is far higher than what is reported to be in commercially produced Aroclor mixtures, where PCB11 constituted up to 0.16% of total mass.

Hornbuckle collaborates with Bill Wombacher, a masters student at UI's College of Engineering; they are working on a piece of equipment that will be used to measure particles in the air.

Dr. Hornbuckle notes that it is possible that PCB11 has been present in the ambient chemical profile for some time. The Chicago metro area experiences elevated background concentrations of PCBs relative to other parts of the country due to the presence of heavy industry in the area prior to the banning of their manufacture in 1977. Previous studies have identified PCBs in the airshed, though no other groups have positively identified PCB11 in their assays. In addition, Dr. Hornbuckle notes that none of the potential precursors to PCB11 are present in large quantities in the local air profile. Dr. Hornbuckle suggests that this indicates a source other than commercial Aroclor products. Given both the widespread geographic distribution and the high concentrations found in residential areas, a possible source of contamination may be current and/or historical use of paint on building interiors and exteriors. Indoor concentrations, and therefore exposures, may be far higher than what was detected outdoors in this study.

Significance:

Dr. Hornbuckle's work chronicles the first identification of PCB11 in an ambient urban atmosphere. The development of the mobile Hi-Vol sample collection system mitigates the logistical difficulties of sampling at multiple remote sites by removing some of the costs associated with installing and maintaining the equipment. It also reduces the labor costs of collecting and transporting samples. Additional study of PCB11 is needed, as much is unknown about this potentially toxic compound. Further areas of study include: positively identifying sources; determining prevalence, if any, in other geographic areas; and investigating if this compound is present in any other matrices or ecosystems.

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

Hu, Dingfei, Keri C. Hornbuckle, and A. Martinez. 2008. Discovery of Non-Aroclor PCB (3,3'-Dichlorobiphenyl) in Chicago Air. Environmental Science and Technology. (http://pubs.acs.org/journals/esthag/) doi:10.1021/es801823r (http://dx.doi.org/10.1021/es801823r) 42:(in press)

Research Brief 168: Zero Valent Iron for Passive Treatment of Acid Rock Drainage

Release Date: 12/03/2008

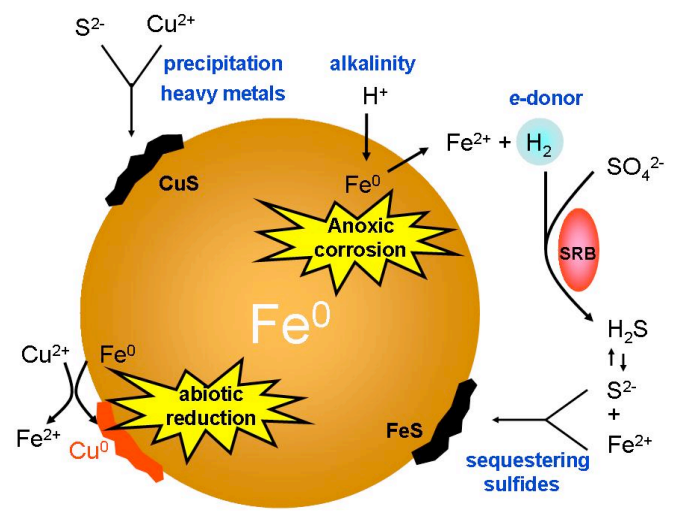
Background:

Abandoned mines and mine tailings from metal ore mining generate large quantities of acidic rock drainage (ARD). ARD is formed by the accelerated weathering of metal-sulfide-rich rocks that are exposed to oxygen by mining activities. The resulting plumes are not only highly acidic (due to sulfuric acid formed from sulfide mineral oxidation), but also contain high concentrations of heavy metals (e.g., Cu, Zn, Pb). ARD lowers the pH of water to levels toxic to aquatic flora and fauna, disrupting the ecosystem at all levels.

Because ARD is typically generated at remote sites where the parties responsible for the contamination are difficult to pinpoint, viable solutions for the treatment of ARD must be low-cost and low-maintenance. Often, passive treatment systems such as constructed wetlands and permeable reactive barriers (PRB) are used. Many passive treatment strategies rely on the activity of sulfate-reducing bacteria (SRB), which utilize sulfate as a terminal electron acceptor in the absence of oxygen. PRBs for treatment of ARD generally use organic substrates as electron donors.

Dr. Jim Field, University of Arizona SBRP, is evaluating zero valent iron (ZVI) as an inexpensive electron donating media in PRBs used for the treatment of ARD. He believes that including ZVI in a PRB could:

- Provide a slow release of electrons that could be used as an energy substrate by sulfate-reducing bacteria.
- Sequester excess sulfide without interfering with the precipitation of heavy metals.
- Generate high levels of alkalinity via anoxic corrosion, raising the pH of ARD.
- Allow for alternative mechanisms of removing heavy metals, such as direct reduction of metals; precipitation of metals due to the greater alkalinity; and adsorption of metals on secondary iron minerals formed from the corrosion of iron.



Mechanisms of zero valent iron during passive treatment of acid rock drainage

Advances:

Dr. Field's group built packed bed columns that contained varying ratiosof sand, compost, crushed limestone, and ZVI. They packed the control column with a mixture of compost and sand; while the treatment column was packed with a mixture of ZVI, compost, and sand. The researchers fed synthetic ARD through the columns, and found that:

1. The treatment column sustained steady sulfate-reducing activity for more than 400 days.
2. The control column sustained sulfate-reducing activity for only 250 days when the biodegradable substrate in the compost was exhausted. This left the sulfate-reducing bacteria with no source of electrons to fuel metabolic processes.

Visual evidence of elemental Cu formation in a packed bed reactor filled with sand and ZVI, and fed with a synthetic ARD of variable Cu2+ concentrations.

The researchers also measured outflow of sulfides from each column. In the control column, sulfate was converted to sulfide and discharged; the treatment column (containing ZVI) reduced approximately twice as much sulfate than the control, but discharged approximately one fourth of the sulfide indicating that excess sulfides were being sequestered.

To examine the impact of ZVI on removal of metals, the researchers added copper to the synthetic ARD mixture. The control column removed nearly as much Cu as the ZVI-containing column for the first 250 days, after which the control removed only 70-80% as much as the ZVI-containing (treatment) column.

Dr. Field also studied the pH of column effluents. From Day 0-200, the synthetic ARD was introduced into the columns at pH 7.3; after Day 200, ARD pH was reduced to 3. Following this change, the pH of control effluent decreased from 7.3 to 6.3, and the pH of the treatment column effluent remained semi-alkaline (pH 9.5), due to the extra alkalinity production by ZVI corrosion.

Significance:

Dr. Field's research demonstrates that ZVI is readily utilized as an electron source by sulfate reducing bacteria. The lab's work demonstrates that the inclusion of ZVI in passive sulfate-reducing systems for ARD treatment may improve their performance. In addition to an increased electron supply, ZVI lowers sulfide discharge, improves the removal of Cu over long-term operation, and results in a greater production of alkalinity.

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

Field, James A. 2005. Zero Valent Iron as an Electron-Donor for Methanogenesis and Sulfate Reduction in Anaerobic Sludge. Biotechnology and Bioengineering. (http://www.interscience.wiley.com/jpages/0006 -3592/) doi:10.1002/bit.20623 (http://dx.doi.org/10.1002/bit.20623) 92:810-819.