

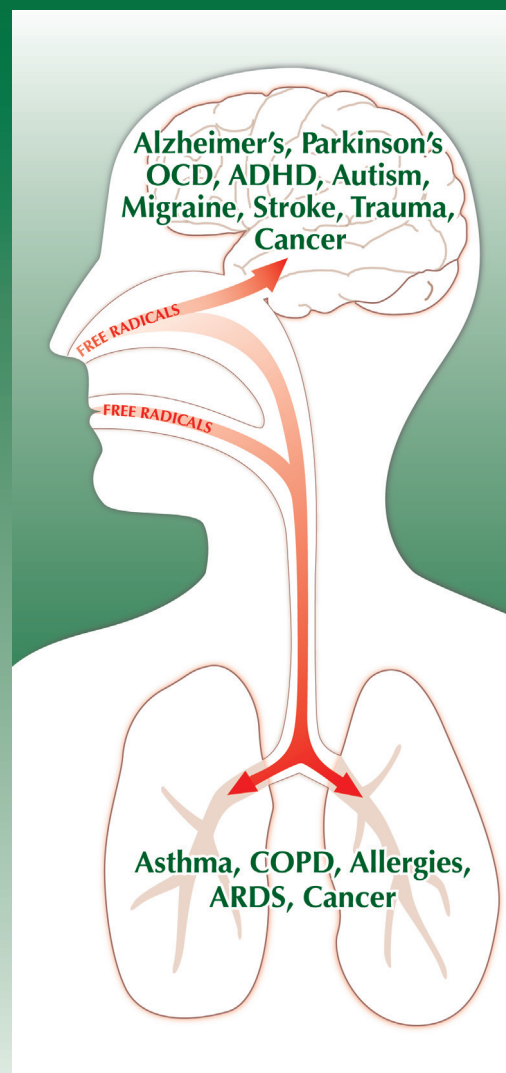
# Center of Excellence in Environmental Toxicology

Fourth Annual Symposium

## Oxidative and Nitrate Stress and Environmental Health

June 4, 2010

Villanova Conference Center



Center of Excellence  
in Environmental Toxicology

FOURTH ANNUAL SYMPOSIUM

**Oxidative and Nitrate Stress  
and Environmental Health**

Villanova Conference Center  
June 4, 2010

Host Institution

CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY  
UNIVERSITY OF PENNSYLVANIA SCHOOL OF MEDICINE



CEET

NIEHS



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# Fourth Annual CEET Symposium

## Oxidative and Nitrate Stress and Environmental Health

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7:30 – 7:55 A.M. **BREAKFAST AND REGISTRATION**

7:55 A.M. Introductory Remarks

**Trevor Penning, Ph.D.**

Director, Center of Excellence in Environmental Toxicology

MORNING SESSION

**Oxidative and nitrate stress and lung and airway disease**

8:00 A.M. **Andrew J. Gow, Ph.D.**

Department of Pharmacology and Toxicology, Rutgers University, Ernest Mario School of Pharmacy  
“Role of nitric oxide and its related redox active molecules in health and disease”

8:30 A.M. **Angela Haczku, M.D., Ph.D.**, Challenge Grant Awardee - NIEHS

“Ozone-induced changes in immune regulation of airway inflammation”

9:00 A.M. **Ian A. Blair, Ph.D.**

“Oxidative stress and 15-hydroxyprostaglandin dehydrogenase-derived lipid mediators”

9:30 A.M. **Clementina Mesaros, Ph.D.**

“Biomarker facility core, CEET”

10:00 – 10:25 A.M. **COFFEE BREAK**

10:30 A.M. **Edward Emmett, M.D., M.S.**

“Translation to the communities: asbestos exposure and the Bo-RIT Superfund Site”

11:15 A.M. Keynote Lecture 1: **Steven Tanenbaum, Ph.D.**

Professor of Chemistry and Toxicology  
Member, MIT Environmental Health Sciences Core Center

“Pathophysiological consequences of nitric oxide and its oxidation products”

12:15 – 1:15 P.M. **LUNCH**

AFTERNOON SESSION

**Oxidative and nitrate stress and neurodegenerative disease**

1:30 P.M. **Harry Ischiropoulos, Ph.D.**

“Oxidative stress and neurodegeneration”

2:00 P.M. **Kelly Jordan-Sciutto, Ph.D.**

“The Nrf2/Keap1 system and neurodegeneration”

2:30 P.M. **Paul Axelsen, M.D.**

“Amyloidogenesis and lipid peroxidation”

3:00 P.M. **Chao-Xing Yuan, Ph.D.**

“Proteomics facility core, CEET”

3:30 – 4:45 P.M. **BREAK AND POSTERS**

4:45 P.M. Keynote Lecture 2: **Tim Greenamyre M.D., Ph.D.**

Professor of Neurology  
Director, Pittsburgh Institute for Neurodegenerative Diseases, University of Pittsburgh  
“Modeling Parkinson’s Disease with a naturally-occurring pesticide”

5:45 P.M. **WINE AND CHEESE RECEPTION**



## Keynote Speakers



**Tim Greenamyre** is Professor and Vice-Chair of Neurology at the University of Pittsburgh School of Medicine, UPMC Endowed Chair & Chief of the Movement Disorders Division, and Director of the Pittsburgh Institute for Neurodegenerative Diseases (PIND) and the American Parkinson Disease Association Advanced Center for Parkinson's Disease Research at the University of Pittsburgh. He is a member of the Scientific Advisory Boards of the Michael J. Fox Foundation and the Parkinson's Disease Foundation. He is the Editor-in-Chief of *Neurobiology of Disease*.

His lab is interested in mechanisms that cause nerve cell death in disorders such as Parkinson's, Huntington's and Alzheimer's diseases. With respect to Parkinson's disease, he is interested in interactions between environmental toxins (natural or man-made) and genes that increase or decrease an individual's susceptibility to developing the disease. The work focuses on mitochondrial impairment, oxidative damage and protein aggregation. In Huntington's disease, the lab focuses on mitochondrial calcium handling and proteomics. The general strategy is to define mechanisms that cause nerve cell death, and then use them as potential "targets" for therapeutic intervention. The lab employs *in vivo* models of neurodegeneration and *in vitro* culture of cells and brain slices to study mechanisms of degeneration with a variety of biochemical, anatomical and physiological techniques.

As Chief of the Movement Disorders Division, Dr. Greenamyre maintains an active clinical practice that is focused on Parkinson's disease, Huntington's disease and related disorders. He is currently listed as one of "The Best Doctors in America" and as one of "America's Top Physicians". In addition to his laboratory research, Dr. Greenamyre participates in a variety of clinical studies and drug trials.



**Steven R. Tannenbaum** is Underwood-Prescott Professor of Toxicology in the Department of Biological Engineering and Professor of Chemistry in the Department of Chemistry at M.I.T. He received his B.S. degree in 1958 and his Ph.D. in 1962, both from M.I.T. and he has been a member of the faculty since 1964. He is a co-founder of the Department of Biological Engineering, the first new academic Department in the MIT School of Engineering in over a generation, and was Co-Director from 1998 to July 2003. Prior to that he was Director of the Division of Toxicology (1996-1998). He was Director of Research of the Cambridge University-MIT Institute, an effort sponsored by the Chancellor of the Exchequer, UK, to develop entrepreneurship in British Universities from July 2003 to July 2004. Dr. Tannenbaum has won many honors and awards, notably election to the Institute of Medicine of the National Academy of Sciences, USA; Fellow of the American Association for the Advancement of Science; Princess Takamatsu Cancer Research Fund Lecturer, and most recently the Chemistry in Cancer Award of the American Association for Cancer Research.

The Tannenbaum laboratory has done pioneering research in several areas, including the discovery of the endogenous formation of nitrogen oxides in humans and other mammals, the development of important biomarkers of human exposure to chemical carcinogens, and the advancement of chemical and spectroscopic methods of detection of chemical adducts to DNA and proteins. His current areas of research are in mechanisms of biochemical activation of chemicals to electrophilic forms that bind to DNA and proteins; application of biomarkers to the molecular epidemiology of disease, the role of endogenous nitric oxide in inflammation, cytotoxicity, and DNA damage, and development of new tools for drug metabolism, pharmacokinetics, and toxicology. Professor Tannenbaum is the author or coauthor of approximately 400 scientific papers, 11 patents, has edited seven books, has helped organize international conferences, and has been on the editorial board of three cancer research and one chemical toxicology journal. At M.I.T., Professor Tannenbaum teaches undergraduate and graduate courses in mechanisms of drug metabolism and toxicity, and case studies in strategies for drug discovery and development.

Dr. Tannenbaum has consulted extensively for the food, chemical, pharmaceutical, and biotechnology industries, has served on many Scientific Advisory Boards, and has been an advisor to US government institutions (NIH, EPA, FDA, NAS/NRC), as well as international agencies (WHO/IARC, FAO, UNDP). He was the Chair of the NAS/NRC Committee to Review the EPA Research Program for Homeland Security.

## MISSION

The Center of Excellence in Environmental Toxicology (CEET) was launched in 2005 and receives grant support from the National Institute of Environmental Health Sciences. It is one of only seventeen designated Environmental Health Science Core Centers in the nation.

The CEET mission is to understand the mechanistic link between environmental exposures and diseases of environmental etiology. Understanding these processes can lead to early diagnosis, intervention and prevention strategies. The end result will be to improve environmental health and medicine in our region.

The CEET is a flexible entity that marshals excellence in basic, translational, patient oriented and population based research in the School of Medicine and Children's Hospital of Philadelphia to facilitate an integrative approach to environmental health/medicine. Although primarily housed in the School of Medicine, the fifty-five CEET Investigators belong to sixteen departments and five schools at the University of Pennsylvania.

The CEET marries its relevant research excellence to diseases of environmental etiology that affect our urban region. The CEET includes an affinity group in Lung and Airway Disease (asthma, lung cancer, mesothelioma, and chronic obstructive pulmonary disease) because of the poor air-quality and air-pollution in our region (ozone, fine particulate matter, allergens, SO<sub>2</sub>, NO<sub>2</sub> and CO emissions). The CEET also has an affinity group in Endocrine and Reproduction Disruption because of the high incidence of adverse pregnancy outcomes that lead to low-weight birth and birth and developmental defects in our region. These organ-based cores are linked to our affinity groups in disease mechanism, which include Oxidative Stress and Oxidative Stress Injury and Gene- Environment Interactions.

The CEET facilitates research by supporting two large facility cores. The Molecular Profiling Core employs toxicogenomic, toxicoproteomic, biomarker and metabolomic approaches to conduct predictive molecular toxicology at a systems-wide level to identify molecular fingerprints of toxicant exposure and response, and disease of environmental etiology. The Integrative Health Sciences Facility Core provides the infrastructure to perform patient and population based environmental health research. It is equipped with the means to conduct human inhalation studies (inhalation chamber-planned opening Fall 2010), epidemiological studies in targeted communities, to access human biospecimens through a CEET virtual biorepository, and the means to conduct study design and biostatistical analysis on genetic and non-genetic projects.

The CEET aims to conduct research relevant to the forty-five Superfund Sites that permeate the region. Studies will elucidate: mechanisms of chemical toxicity; exposure levels, risk assessment and health hazard; bioremediation approaches; and effects on ecosystems and biodiversity.

The Community Outreach and Education Core (COEC) works with and disseminates research findings to select local communities to empower them with new knowledge so that they are better informed to tackle issues of health disparities and environmental justice. To improve the environmental health of these and similar affected communities, the COEC is actively involved in the education of health care professionals (Residency Program in Occupational and Environmental Health, Nursing concentration in Occupational and Environmental Health, and Masters of Public Health Programs).

The COEC also disseminates its mission and its research findings to all stakeholders including community organizations, local, state and federal officials and agencies (Pennsylvania Department of Health, Pennsylvania Department of Environmental Protection, Environmental Protection Agency) to affect change in environmental health and public health policies.

# CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

## University of Pennsylvania School of Medicine

### ADMINISTRATIVE CORE

Director: Trevor Penning, Ph.D.

Deputy Director: Reynold Panettieri, M.D.

#### **Affinity Group I**

OXIDATIVE STRESS AND  
OXIDATIVE STRESS INJURY

Co-Leader: Ian Blair, Ph.D.  
Co-Leader: Harry Ischiropoulos, Ph.D.  
Joseph Baur, Ph.D.  
Michael Beers, M.D.  
Jeffrey Field, Ph.D.  
Aron Fisher, M.D.  
Garret FitzGerald, M.D.  
Benoit Giasson, Ph.D.  
Toshinori Hoshi, Ph.D.  
Vladimir Muzykantov, M.D., Ph.D.  
Trevor Penning, Ph.D.  
Richard Schultz, Ph.D.  
Rebecca Simmons, M.D.  
Andrew Strasser, Ph.D.  
Stephen Thom, M.D., Ph.D.

#### **Affinity Group II**

ENDOCRINE/REPRODUCTION  
DISRUPTION

Co-Leader: George Gerton, Ph.D.  
Co-Leader: Samuel Parry, M.D.  
Kurt Barnhart, M.D, M.S.C.E.  
Marisa Bartolomei, Ph.D.  
Samantha Butts, M.D., M.S.C.E.  
Ted Emmett, M.D., M.S.  
Norman Hecht, Ph.D.  
Jianghong Liu, Ph.D., R.N.  
Mary Mullins, Ph.D.  
Katherine Nathanson, M.D.  
Trevor Penning, Ph.D.  
Richard Schultz, Ph.D.  
Rebecca Simmons, M.D.  
Wenchao Song, Ph.D.

#### **Affinity Group III**

LUNG AND AIRWAY DISEASE

Co-Leader: Michael Beers, M.D.  
Co-Leader: Steve Albelda, M.D.  
Andrea Apter, M.D., M.Sc.  
Tyra Bryant-Stephens, M.D.  
Jason Christie, M.D., M.S.C.E.  
Melpo Christofidou-Solomidou, Ph.D.  
Richard Doty, Ph.D.  
Angela Haczku, M.D., Ph.D.  
James Kreindler, M.D.  
Vera Krymskaya, Ph.D.  
Frank Leone, M.D.  
Rey Panettieri, M.D.  
Trevor Penning, Ph.D.  
Anil Vachani, M.D.

#### **Affinity Group IV**

GENE-ENVIRONMENT INTERACTIONS

Co-Leader: Tim Rebbeck, Ph.D.  
Co-Leader: Alexander S. Whitehead, D.Phil.  
Marisa Bartolomei, Ph.D.  
Ian Blair, Ph.D.  
Michael Burczynski, Ph.D.  
Jinbo Chen, Ph.D.  
Vivian Cheung, Ph.D.  
Jason Christie, M.D., M.S.C.E.  
Hakon Hakonarson, M.D., Ph.D.  
John Hogenesch, Ph.D.  
Todd Lamitina, Ph.D.  
Caryn Lerman, Ph.D.  
Hongzhe Li, Ph.D.  
Jennifer Pinto-Martin, Ph.D., M.P.H.  
Katherine Nathanson, M.D.  
Trevor Penning, Ph.D.  
Sarah Tishkoff, Ph.D.

# CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

## University of Pennsylvania School of Medicine

### MOLECULAR PROFILING CORE

Director: Ian Blair, Ph.D.

*Toxicogenomics*

Associate Director: Don Baldwin, Ph.D.

Associate Director: John Tobias, Ph.D.

*Toxicoproteomics*

Associate Director: Chao-Xing Yuan, Ph.D.

*Biomarker*

Associate Director: Clementina Mesaros, Ph.D.

### INTEGRATED HEALTH SCIENCES FACILITY CORE

Director: Rey Panettieri, M.D.

*Human Studies Design and  
Performance Services*

Associate Director: Michael Sims, M.D., M.S.C.E.

*Population Exposure Services*

Associate Director: Ted Emmett, M.D., M.S.

*CEET Biorepositories*

Associate Director: Michael Feldman, M.D., Ph.D.

*Biomedical Informatics Group*

Associate Director: J. Richard Landis, Ph.D.

*Biostatistics*

Associate Director: Andrea Troxel, Ph.D.

Genetics Statistician: Mingyao Li, Ph.D.

### COMMUNITY OUTREACH AND EDUCATION

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Deputy Director: Richard Pepino, M.S.

Andrea Apter, M.D., M.Sc.

Charles Branas, Ph.D.

Tyra Bryant-Stephens, M.D.

Pamela Dalton, Ph.D.

Jeffrey Field, Ph.D.

Ira Harkavy, Ph.D.

Marilyn Howarth, M.D.

Howard Kunreuther, Ph.D.

Jianghong Liu, Ph.D., R.N.

Judith McKenzie, M.D., M.P.H.

Kevin Osterhoudt, M.D., M.S.C.E.

Trevor Penning, Ph.D.

Jennifer Pinto-Martin, Ph.D., M.P.H.

Alexander S. Whitehead, Ph.D.

## Lung and Airway Disease

### L1 Alterations in multimeric structure of surfactant protein D as a biomarker for lung injury and inflammation in humans

*Atochina-Vasserman, Elena N (Pulmonary and Critical Care Division, Dept of Medicine, University of Pennsylvania)*

*Hohlfeld, Jens (Fraunhofer ITEM, Hannover, Germany)*

*Sims, Karen (Division of Infectious Disease, Dept of Medicine, University of Pennsylvania)*

*Guttentag, Susan H (Division of Neonatology Dept of Pediatrics Childrens Hospital of Philadelphia)*

*Gow, Andrew J (Dept of Pharmacology & Toxicology, Rutgers University, Piscataway, NJ)*

*Beers, Michael F (Pulmonary and Critical Care Division, Dept of Medicine, University of Pennsylvania)*

Surfactant Protein D quaternary structure and function has been shown to be modulated in part through post-translational modification by NO and oxidants. Asthma, Hermansky Pudlak Syndrome, and chronic rejection of lung allografts are all associated with amplification of inflammatory signals in the distal lung. We hypothesized that alterations in structure and function of SP-D would be associated with these diseases in humans. SP-D expression in BAL from 3 representative patient populations and controls was evaluated by Western blotting, native PAGE, and Biotin Switch assay. BAL from each disease group demonstrated the presence of S-nitrosylated SP-D and significant disruption of the multimeric native structure. In half of the asthmatic patients, a covalently cross-linked SP-D isoform was detected after segmental challenge which correlated positively with BAL eosinophil counts. Cross-linked forms of SP-D were also recovered from patients undergoing chronic lung allograft rejection but were not detected in BAL from HPS patients. These results suggest that SP-D isoforms, in addition to contributing to local immunomodulation in the lung, are potential candidates for biomarkers of pulmonary inflammation. (*Funded by NIH HL64520*)

### L2 20-HETE mediates ozone-induced, neutrophil-independent airway hyper-responsiveness in mice

*Cooper, P. R.; Mesaros A.C.; Zhang J., Christmas P., Stark C. M., Douaidy K., Mittelman M. A., Soberman R. J., Blair I. A., Panettieri R. A. (Department of Medicine and the Airways Biology Initiative, University of Pennsylvania School of Medicine)*

**Background:** Ozone, a pollutant known to induce airway hyper-responsiveness (AHR), increases morbidity and mortality in patients with obstructive airway diseases and asthma. We postulate oxidized lipids mediate *in vivo* ozone-induced AHR in murine airways.

**Methodology/Principal Findings:** Male BALB/c mice were exposed to ozone (3 or 6 ppm) or filtered air (controls) for 2 h. Precision cut lung slices (PCLS; 250 microm thickness) containing an intrapulmonary airway (approximately 0.01 mm<sup>2</sup>) lumen area were prepared immediately after exposure or 16 h later. After 24 h, airways were contracted to carbachol (CCh). Log EC(50) and E(max) values were then calculated by measuring the airway lumen area with respect to baseline. In parallel studies, dexamethasone (2.5 mg/kg), or 1-aminobenzotriazol (ABT) (50 mg/kg) were given intraperitoneal injection to naïve mice 18 h prior to ozone exposure. Indomethacin (10 mg/kg) was administered 2 h prior. Cell counts, cytokine levels and liquid chromatography-mass spectrometry (LC-MS) for lipid analysis were assessed in bronchoalveolar lavage (BAL) fluid from ozone exposed and control mice. Ozone acutely induced AHR to CCh. Dexamethasone or indomethacin had little effect on the ozone-induced AHR; while, ABT, a cytochrome P450 inhibitor, markedly attenuated airway sensitivity. BAL fluid from ozone exposed animals, which did not contain an increase in neutrophils or interleukin (IL)-6 levels, increased airway sensitivity following *in vitro* incubation with a naïve PCLS. In parallel, significant increases in oxidized lipids were also identified using LC-MS with increases of 20-HETE that were decreased following ABT treatment.

**Conclusions/Significance:** These data show that ozone acutely induces AHR to CCh independent of inflammation and is insensitive to steroid treatment or cyclooxygenase (COX) inhibition. BAL fluid from ozone exposed mice mimicked the effects of *in vivo* ozone exposure that were associated with marked increases in oxidized lipids. 20-HETE plays a pivotal role in mediating acute ozone-induced AHR.

## Lung and Airway Disease

### L3 Metabolism of natural and synthetic glucocorticoids by aldo-keto reductase 1 family enzymes

*Jin, Yi (University of Pennsylvania)*

The sequential actions of AKR1D1 (5beta-steroid reductase) and AKR1C1-4 (ketosteroid reductases) are implicated in liver cortisol metabolism. The following substrate/enzyme systems were investigated: cortisol/AKR1D1, 5alpha- and 5beta-dihydrocortisol/AKR1C1-4, and cortisol/AKR1C1-4. Reaction products were identified and kinetic constants were determined. AKR1D1 reduced cortisol to 5beta-dihydrocortisol. The reaction of 5alpha- and 5beta-dihydrocortisol with all four AKR1C enzymes yielded the respective 3alpha-tetrahydrocortisol as the main product. AKR1C4 displayed the highest catalytic efficiency with both dihydrocortisols among all AKR1Cs and is identified as the principal enzyme for the formation of tetrahydrocortisols in liver. Low turnover rates were observed for the 20-keto reduction of cortisol by AKR1Cs. Results are consistent with AKR enzymes playing a critical role in hepatic cortisol metabolism. In addition, AKR1D1 also acted on asthma drugs budesonide and flunisolide with catalytic efficiencies 6-10 fold lower than that of cortisol, suggesting AKR1 family members may also be involved in the systemic metabolism of synthetic glucocorticoids. (*Supported by a pilot project fund from NIH grant P30 ES015857 and R01-DK47015*)

### L4 A Model of ozone-induced allergic asthma exhibits altered Surfactant Protein-D (SP-D) structure, increased release of eosinophil- and dendritic cell specific mediators, and activation of macrophages and dendritic cells of the airways

*Koziol-White, Cynthia J (Pulmonary, Allergy and Critical Care Division, University of Pennsylvania)*

*Forbes, Lisa R. (Children's Hospital of Philadelphia, University of Philadelphia)*

*Ducka, Blerina (Pulmonary, Allergy and Critical Care Division, University of Pennsylvania)*

*Fehrenbach, Melane (Pulmonary, Allergy and Critical Care Division, University of Pennsylvania)*

*Kierstein, Sonja (Pulmonary, Allergy and Critical Care Division, University of Pennsylvania)*

*Sharma, Satish K. (Pulmonary, Allergy and Critical Care Division, University of Pennsylvania),*

*Haczku, Angela (Pulmonary, Allergy and Critical Care Division, University of Pennsylvania)*

**Rationale:** O<sub>3</sub> may promote inflammation in the asthmatic airways by damaging the immunoprotective function of the SP-D molecule.

**Methods:** Balb/c mice were sensitized and challenged with *Aspergillus fumigatus* (Af) and subsequently exposed to O<sub>3</sub>. Bronchoalveolar lavage (BAL) was assessed for SP-D by native PAGE and cytokine/chemokine profile by ELISA. BAL cell SP-D binding, activation and effects of SP-D on dendritic cells were investigated by immunocytochemistry and FACS.

**Results:** Treatment of SP-D<sup>-/-</sup> BAL cells with recombinant SP-D showed a dose-dependent binding and colocalization with a signal inhibitor membrane molecule, SIRP-alpha. SP-D inhibited dendritic cell maturation and TNF-alpha release *in vitro*. O<sub>3</sub> exacerbated airway inflammation in Af-sensitized mice. TNF-alpha and IL-6 release tracked with expression of CD91 on MHCII<sup>+</sup> cells. O<sub>3</sub> induced appearance of abnormal oligomeric SP-D in BAL fluid.

**Conclusions:** Increased CD91 expression makes innate immune cells susceptible to proinflammatory stimulation. O<sub>3</sub>-induced conformational changes in SP-D may confer a loss of ability to bind SIRP-alpha. Together these effects can result in loss of SP-D immunoprotection during O<sub>3</sub>-induced exacerbation of allergic airway changes.

## Lung and Airway Disease

### L5 Aldo-keto reductases protect lung epithelial cells from polycyclic aromatic hydrocarbons at the expense of increased oxidative stress

*Abedin, Zahidur* (University of Pennsylvania)

*Sen, Sushmita* (University of Pennsylvania),

*Field, Jeffrey* (University of Pennsylvania)

Tobacco exposure stimulates genes that are likely to be involved in the metabolism of tobacco and its combustion products. Four induced genes are aldo-keto reductases (AKRs). AKRs act on a range of substrates including polycyclic aromatic hydrocarbons (PAH). AKRs convert PAH to form o-quinones. Alternatively, PAHs are metabolized to (+)-anti-BPDE by the combined action of P4501A1/1B1 and epoxide hydrolase. To address the role of AKRs in PAH toxicity, we compared the toxicities of PAH metabolites and the effects of overexpressing AKR 1A1 in lung cells. We find that (+)-anti-BPDE and BP,-7,8-diol are toxic to A549 cells at concentrations with an IC<sub>50</sub> of ~2 μM. In contrast, we found that the B[a]P-7,8-dione was about 10-fold less toxic to A549 cells with an IC<sub>50</sub> > 20 μM. However, B[a]P-7,8-dione treatment generated reactive oxygen species (ROS) in A549 cell as previously reported. We also observed that overexpression of AKR 1A1 in H358 cells protected cells 2-10 fold from the toxic effects of BP, 7,8-diol. These data suggest that overexpression of AKRs may protect lung cancer cells from the acute toxic effects of PAH at the expense of increasing the burden of oxidative stress.

### L6 Persistent oxidative stress in H358 cells treated with PAH-o-quinones

*Basu, Sankha S* (University of Pennsylvania School of Medicine, Dept of Pharmacology, CEET)

*Gelhaus, Stacy G* (University of Pennsylvania School of Medicine, Dept of Pharmacology, CEET)

*Huang, Meng* (University of Pennsylvania School of Medicine, Dept of Pharmacology, CEET)

*Penning, Trevor* (University of Pennsylvania School of Medicine, Dept of Pharmacology, CEET)

*Blair, Ian* (University of Pennsylvania School of Medicine, Dept of Pharmacology, CEET)

Metabolism of benzo[a]pyrene by aldo-keto-reductases (AKRs) results in the formation of B[a]P-7,8-dione (BPQ), which has been shown to undergo futile redox cycling resulting in reactive oxygen species (ROS) formation in lung cells. In this study, H358 cells were treated with BPQ for a range of doses and incubation times. Liquid chromatography-mass spectrometry was used to measure changes in glutathione/glutathione disulfide levels and monitor BPQ levels in cells and media after treatment. Intracellular ROS generation was monitored using flow cytometry. We found that while BPQ rapidly disappeared from both media and cells, increased oxidative stress persisted in cells for twenty-four hours. Furthermore, a significant fraction of BPQ was associated with proteins and resulted in persistent autofluorescent products that were visualized by fluorescence microscopy and quantified by flow cytometry. These long-lived BPQ adducts represent a potential intracellular ROS-generating reservoir and may play a significant role in smoking-related oxidative stress and carcinogenicity.

## Lung and Airway Disease

### L7 Metabolism of benzo[a]pyrene-7,8-dione in human lung cells

**Huang, Meng** (Department of Pharmacology, University of Pennsylvania School of Medicine),

**Liu, Xiaojing** (Department of Pharmacology, University of Pennsylvania School of Medicine),

**Blair, Ian A.** (Department of Pharmacology, University of Pennsylvania School of Medicine),

**Penning, Trevor M.** (Department of Pharmacology, University of Pennsylvania School of Medicine)

Benzo[a]pyrene-7,8-dione(B[a]P-7,8-dione)is produced in human lung cells by the oxidation of B[a]P-7,8-trans-dihydrodiol catalyzed by aldo-keto reductases (AKRs). However, information relevant to the *in vivo* metabolism of B[a]P-7,8-dione in these cells is lacking. We studied the metabolic fate of 2.0  $\mu$ M [3H]-B[a]P-7,8-dione in human bronchoalveolar H358 cells, human lung adenocarcinoma A549 cells, and immortalized human bronchial epithelial HBEC-KT cells. In these three cell lines, radioactivity was distributed in the organic phase and aqueous phase of medium, as well as in the cell lysate and cell pellets. After acidification of the medium, several metabolites of [3H]-B[a]P-7,8-dione were detected in the organic phase of the medium by HPLC-UV-RAM. The structures of B[a]P-7,8-dione metabolites in the cell lines were identified as GSH-B[a]P-7,8-dione, N-acetyl-L-cysteine-B[a]P-7,8-dione, N-acetyl-L-cysteine-7,8-dihydroxy-B[a]P and O-methylated -7,8-dihydroxy-B[a]P by LC-MS/MS and were subsequently validated by comparison to authentic synthesized standards. (Supported by 1R01-CA39504 to TMP)

### L8 O-methylation of PAH catechols as a detoxification route for PAH o-quinones

**Zhang, Li** (CEET and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania)

**Gelhaus, Stacy** (CEET and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania)

**Blair, Ian A.** (CEET and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania)

**Penning, Trevor M.** (CEET and Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania)

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants found in tobacco smoke, residues of fossil fuel burning and are associated with the causation of lung cancer. PAH require metabolic activation to elicit their deleterious effects. Cytochrome P450s and aldo-keto reductases (AKRs) are two major activation pathways of PAH trans-dihydrodiols. The formation of redox active and electrophilic o-quinones catalyzed by AKRs leads to the generation of reactive oxygen species and adduct formation with DNA etc. Phase II enzymes can potentially catalyze O- glucuronidation, sulfation and methylation of the PAH catechol and prevent redox-cycling of the PAH o-quinone. We explored whether catechol O-methyl transferase (COMT) could detoxify 7,8-dihydroxy-benzo[a]pyrene formed from the reduction of benzo[a]pyrene-7,8-dione (BPQ). BPQ was reduced to PAH catechol by dithiothreitol under anaerobic conditions and then further O-methylated by porcine COMT in the presence of [3H] S-adenosyl methionine as a methyl group donor. The formation of the O-methylated catechol was detected by both HPLC-RAM-UV and quantified by scintillation counting. Reactions were also replicated using unlabeled cofactor and the O-methylated products were characterized by LC-MS. The kinetics of o-methylation was monitored and a  $K_m$  of 4.9 $\mu$ M and  $V_{max}$  of 3.57 nmol/min/mg was observed using the porcine enzyme. In conclusion, O-methylation is a feasible phase II reaction for the detoxification of PAH o-quinones (Supported by 1R01-CA35904 and P30-ES013587.)

## Lung and Airway Disease

### L9 **Beas2B cells as a model of benzo[a]pyrene metabolism in noncancerous human lung: a functional genomics approach toward understanding metabolic consequences of gene expression**

*Kushman, Mary E. (CEET and Department of Pharmacology, University of Pennsylvania School of Medicine)*

*Penning, Trevor M. (CEET and Department of Pharmacology, University of Pennsylvania School of Medicine)*

A comparative functional genomics study of basal and induced AKR and P450 expression in Beas2B cells, with implications for metabolic consequences of gene expression, was performed. Real-time PCR analysis of P450 and AKR expression revealed detectable P4501A1 and 1B1 mRNA transcripts that were induced after treatment with TCDD. Functional P450 activity was also present. AKR1C transcripts, while detectable, were 100-fold less; no functional AKR1C activity was detected. Metabolic profiling via radiometric RP-HPLC of 1 micromolar [3H]-B[a]P and [3H]-( $\pm$ )-B[a]P-7,8-dihydrodiol was performed in naive and TCDD-induced Beas2B cells. [3H]-B[a]P metabolism in Beas2B cells resulted in B[a]P-9,10-diol as the predominant daughter metabolite. [3H]-B[a]P-7,8-dihydrodiol was metabolized to the four B[a]P-tetraols. However, TCDD was without influence on the metabolic profile of either B[a]P or its 7,8-dihydrodiol. The AKR product B[a]P-7,8-dione was not detected. Taken together, the results demonstrate that Beas2B cells are metabolically competent for PAH biotransformation, but do not present evidence for the P4501A1 or 1B1 and/or the AKR pathway involved in B[a]P and B[a]P-7,8-diol metabolism. (*Supported by 1R01-ES015857.*)

### L10 **Paradoxical down-regulation of benzo[a]pyrene-mediated DNA-adduct formation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in human lung cells**

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, environmental pollutants. Benzo[a]pyrene (B[a]P), a prototypic PAH, is often used to elucidate the pathways of metabolic activation. Metabolism of B[a]P by cytochrome P450 (CYP) 1A1/1B1 and epoxide hydrolase results in the formation of the ultimate carcinogen, (+)-anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-B[a]P (B[a]PDE). B[a]PDE is able to translocate to the nucleus where it forms DNA-adducts, primarily (+)-anti-trans-B[a]PDE-N2-dGuo (B[a]PDE-dGuo). Typically, pre-treatment of cells with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) will induce CYP1A1/1B1 expression through the aryl hydrocarbon receptor (AhR) pathway thus causing an increase in B[a]PDE-dGuo formation. Unexpectedly, TCDD induction caused a decrease in B[a]PDE-dGuo formation versus the untreated, control human bronchoalveolar, H358 cells. Further investigation revealed that the TCDD-mediated decrease in B[a]PDE-dGuo simultaneously occurs with an increase in B[a]PDE-glutathione-adduct formation. This study reveals that the subtle balance between activation and detoxification of B[a]P in lung cells. (*Research supported by NIH 5F32ES016683.*)

# Oxidative Stress and Oxidative Stress Injury

## O1 Lipoxygenase metabolites from cyclooxygenases

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There has been enormous interest in cyclooxygenase (COX)-derived eicosanoids as biologically active mediators of physiological processes. However, eicosanoids derived from the lipoxygenase (LOX) activity of COXs have been largely ignored. Numerous studies of purified COX-1 and COX-2 enzymes have shown that the LOX-derived 11(R)-hydroxyeicosatetraenoic acid (HETE), 15(S)-HETE, and 15(R)-HETE metabolites are formed in relatively high amounts. They are formed by reduction of the corresponding hydroperoxyeicosatetraenoic acids (HPETES). However decomposition of the HPETES before they are reduced could potentially result in the formation of reactive bifunctional electrophiles. We developed a chiral LC-MS for analysis of enantiomers derived from the bifunctional electrophiles 4-hydroperoxy-2(E)-nonenal and 4-hydroxy-2-(E)-nonenal and shown that they are formed with remarkable enantioselectivity from 15-HPETES. However, homolytic decomposition of 11(R)-HPETE resulted in the formation of racemic mixtures. This provided insight into mechanisms of HPETE decomposition pathways and suggest provided a means to monitor enantioselective formation of intracellular reactive bifunctional electrophiles-derived glutathione-adducts.

## O2 Absolute quantification of oxidative modifications on apoA-I protein by stable isotope dilution LC-MRM/MS assays

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Apolipoprotein A-I (apoA-I), the major protein in High Density Lipoprotein (HDL) protects against atherosclerosis by removing cholesterol from arterial walls. Oxidative modifications mediated by myeloperoxidase (MPO) leading to nitration/chlorination on tyrosine (Y) residues at positions 192 or 166 in apoA-I are known to inhibit this ability during atherosclerosis progression. However, apoA-I also contains additional tyrosine residues at positions 18, 29, 100, 115 and 236, and it is not clear if any of these residues also undergo oxidative modifications, and their biological implication in atherosclerosis. Stable isotope dilution liquid chromatography/tandem mass spectrometry method was utilized to study the oxidative modifications on tyrosine residues, Y18, Y29, Y100, Y115, Y166, Y192 and Y236, in apoA-I.

## Oxidative Stress and Oxidative Stress Injury

### O3 **Dietary advanced glycation endproducts and sleep apnea oxygenation are independent risk factors for oxidative stress in the hippocampus**

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In this series of studies we examined whether lowering dietary advanced glycation endproducts (AGE) simply by reducing food preparation temperatures/cooking times could lessen oxidative injury from sleep apnea.

Mice (8 wks) were fed rodent chow cooked so that so that AGE content was 1750 U/mg food in low-AGE food or 12,500 U/mg reg-AGE food. At 12 wks of age, mice were subjected to 8 weeks of intermittent hypoxia (IH) or sham hypoxia modelling 40/hr sleep apnea oxygenation fluctuations. Mice remained on the diets throughout the experiment with brains then processed for immunohistochemistry and Perl's iron stain.

Low-AGE diet reduced 3-nitrotyrosine (3-NT) and 8-hydroxyguanosine labelling (8-HG) and iron deposition throughout the hippocampus, in neurons and microglia. In reg-AGE and low-AGE fed mice, exposure to intermittent hypoxia resulted in further increased in 3-NT, without further changes in iron or 8-HG. Moreover, intermittent hypoxia resulted in pronounced 3-NT labelling in microglia throughout the hippocampus.

Lowering dietary AGE by reducing cooking lessens nitrosative stress and iron deposition in the hippocampus in a model of sleep apnea.

## Gene-Environment Interactions

### G1 Changes in folate/homocysteine phenotype in response to anti-folate drugs

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Elevated homocysteine (Hcy) is a risk factor for several pathologies, such as stroke, certain cancers, and rheumatoid arthritis (RA); many of these pathologies are also associated with environmental agents, such as tobacco smoke. Hcy levels are affected by functional polymorphisms of enzymes involved in folate/Hcy metabolism and the availability of certain vitamins. Treatment with anti-folate drugs is a useful surrogate for studying the effects of environmental agents on folate/Hcy phenotype. This project explores the effects of treatment with methotrexate (MTX), an anti-folate drug used to treat RA, in combination with methylenetetrahydrofolate reductase (MTHFR) 677C>T genotype on folate/Hcy phenotype. To examine the association between genotype, phenotype, environment and disease state, patients with RA were recruited to attend visits prior to and 24 weeks after starting MTX treatment. Overall, MTHFR 677C>T genotype had a significant effect on the changes from baseline to 24-weeks in plasma folate and several intracellular forms of folate. Understanding the relationship between genotype, phenotype, and environment will facilitate development of genetic and biomarker tests for a range of diseases with environmental influences.

## Biomarker

### B1 A new liquid chromatography/mass spectrometry method for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine

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4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a carcinogenic nitrosamine produced upon curing tobacco. It is present in tobacco smoke and undergoes metabolism to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in the lungs. NNAL has been shown to be a strong lung carcinogen in several animal studies and recently has emerged as a candidate biomarker for exposure to environmental tobacco smoke (ETS). The ability to conduct validated analyses of free and conjugated NNAL in human urine is important in order to assess inter-individual differences in lung cancer risk from exposure to cigarette smoke. Our study describes a novel derivatization procedure resulting in the formation of a pre-ionized NNAL derivative. The increased sensitivity arising from this derivative makes it possible to analyze free NNAL in only 0.25 mL urine by stable isotope dilution liquid chromatography electrospray ionization multiple reaction monitoring mass spectrometry (SID-LC-ESI -MRM/MS). (We gratefully acknowledge support of grants UO1 ESO16004 and P30 ESO13508).

### B2 Analysis of urinary isoprostanes by chiral liquid chromatography-electron capture atmospheric pressure chemical ionization/mass spectrometry

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Isoprostanes (IsoPs) are oxidation products of arachidonic acid, which were originally discovered as artifacts that were present in stored plasma samples. Liquid chromatography-electron capture atmospheric pressure chemical ionization/mass spectrometry (LC-ECAPCI/MS) provides a 25 to 100-fold increase in sensitivity for eicosanoids when compared with conventional APCI/MS methods. Furthermore, analyses can be conducted using normal phase mobile phases and chiral columns without loss of sensitivity when compared with conventional reversed-phase LC. This makes it possible to separate complex mixtures of eicosanoids using chiral normal phase LC columns and to conduct quantification with very high sensitivity and specificity using stable isotope dilution methodology. This methodology has now been applied to the analysis of isoPs in order to determine whether they can be excreted in urine of tobacco smokers and non-smokers as glucuronide conjugates. (We gratefully acknowledge the support of grant P30ES0135080 from the National Institute of Health.)

## Biomarker

### B3 Analysis of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine by liquid chromatography/mass spectrometry

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**Phillips, Laura** (Agilent Technologies, Wilmington, DE)

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7,8-Dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dGuo) is a biomarker of oxidative DNA damage but its urinary analysis has been difficult due to numerous methodological problems. dGuo present in urine can be easily oxidized during sample preparation and would lead to elevated levels of 8-oxo-dGuo. A highly specific and sensitive method based on stable isotope dilution liquid chromatography (LC)-multiple reaction monitoring (MRM)/mass spectrometry (MS) has been developed. This optimized method was then applied to the analysis of the healthy smokers' and non-smokers' urine samples. We acknowledge Agilent Technologies for the kind loan of the 6460 Triple quad. (We gratefully acknowledge the support of grants UO1ES016004 and P30ES0135080 from the National Institute of Health.)

### B4 Chirality study of homolytic decomposition metabolites from hydroperoxides

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Lipid hydroperoxides undergo homolytic decomposition into electrophilic metabolites, which react with DNA to produce DNA adducts. These modifications of DNA are thought to be associated with the etiology of aging such as cardiovascular disease, cancer and neurodegeneration. Lipid hydroperoxides can be formed either enzymatically or non-enzymatically. The lipid hydroperoxydes are highly reactive and are usually reduced to the corresponding hydroxide lipids or decompose through transition-metal-ion or vitamin C-mediated pathways to provide alpha,beta-unsaturated aldehydes. The chirality of the metabolites indicates the mechanism of the decomposition. 4-Hydroperoxy-2(E)-nonenal (HPNE) and hydroxy-nonenal (HNE) are two of the most studied decomposition products. We investigated the chirality of the metabolites formed from enantiomerically pure hydroperoxides derived from arachidonic acid (AA). HNE and HPNE metabolized from 11-(R)-HPETE were found to be racemates. While HNE and HPNE produced from 15-(S)-HPETE and 15-(R)-HPETE retained the chirality. Mechanisms are proposed for the transformations.

# Toxicoproteomics

## T1 Proteomics Core Facility

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*Blair, Ian (University of Pennsylvania)*

The Proteomics Core Facility has been supported by multiple centers and institutions: CEET, ACC, ITMAT, SOM. The resource is committed to providing CEET members with sophisticated proteomics methodologies including protein separation, identification, quantitation, modification analysis and bioinformatics support. This is achieved by offering consultation, assisting investigators in evaluating and designing research projects that effectively incorporate the Core Facility's technologies, and providing investigators with cost-effective access to highly technical services. The Core Facility provides following services:

1. Enzyme digestions such as trypsin, chymotrypsin for protein digestion.
2. NanoLC/nanospray/MS/MS for peptide separation and sequencing.
3. Database searches with various software packages such as Sequest, Mascot, Scaffold.

The core facility submitted a shared instrument grant proposal to NIH for the acquisition of an LTQ Orbitrap for quantitative proteomics. Based on the positive review of the original proposal, we anticipate that the re-submission will be successful.

## Community Outreach and Education Core

### C1 Development of methods for information retention and sharing for community-based participatory research

Study of serum biomarkers of polyfluoroalkyl compounds in young girls

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*Barg, Fran* (Mixed Methods Research Laboratory, University of Pennsylvania)

*Britt, Dahlberg* (Mixed Methods Research Laboratory, University of Pennsylvania)

*Saeber, Muzaffar* (University of Pennsylvania Health System)

*Rosana, Schafer* (University of West Virginia)

*Wolf, Maria* (Center of Excellence in Environmental Toxicology, University of Pennsylvania)

*Jody, Roberts* (Chemical Heritage Foundation)

*Gregory, Cooke* (West Ambler Civic Association)

Environmental health issues cut across divisions between academic and community interests as well as academic disciplines. Devising methods for addressing environmental influences on the progression of human disease requires innovative ways of working across these divisions. This poster outlines existing barriers to community-based participatory research in environmental health, and presents the methods we use in our community partnership to address these barriers and develop new paradigms. Our objectives are to develop sustainable sources of information, provide access to the research team, and facilitate understanding across disciplines. Our methods include using Dropbox to facilitate information sharing, weekly phone meetings, walking tours, and training workshops. We explain how these methods facilitate creativity and respectful collaboration, which allow us to reflect upon the different lessons we bring to investigating our shared research questions. We argue that this approach facilitates a process for interdisciplinary community partnership that helps us confront the assumptions of our own fields and develop new paradigms to resolve ongoing discrepancies that maintain environmental injustice and health disparities.

### C2 Melamine and cyanuric acid do not interfere with Bradford and Ninhydrin assays for protein determination

*Field, Anjalie* (University of Pennsylvania)

*Field, Jeffrey* (University of Pennsylvania)

In the fall of 2007, pet food contaminated with melamine and cyanuric acid caused kidney stones in thousands of animals. In the summer of 2008, a more serious outbreak of adulterated dairy food resulted in the deaths of six infants and sickened about 290,000 children in China. In all cases, melamine was likely added to inflate the apparent protein content of the foods. To determine if we could measure protein without interference from melamine and cyanuric acid we tested these compounds in the Bradford and Ninhydrin assays, two common dye-based assays for protein determination. Neither compound was detected in the Ninhydrin and Bradford assays at concentrations of >100 ug/ml. For validation, assays were run on cat food and reconstituted milk powder. The addition of melamine or cyanuric acid to reconstituted milk did not affect the Bradford readings. The protein concentrations obtained for reconstituted milk powder were as expected, but those for the cat food were 10–30-fold lower, due to its low solubility. We conclude that dye-binding assays can be employed to detect protein in food without interference from melamine and cyanuric acid, thus reducing the incentive to use them as additives.

## Community Outreach and Education Core

### **C3 The Teen Research and Education in Environmental Science (TREES) summer program for high school students**

*Field, Jeffrey (University of Pennsylvania)*

In 2007, the Center for Excellence in Environmental Toxicology launched a community outreach education program for high school students called the Teen Research and Education in Environmental Science (TREES) summer program. The TREES program is a unique hands-on research experience for high school students to introduce them to laboratory science. The program begins with two weeks of structured laboratory exercises to teach basic lab techniques such as pipeting, weighing, microbial techniques, and several spectrophotometric-based assays. This sets the stage for what is the most unique aspect of the program: an individually guided research project on a topic chosen by the students. Projects have earned them numerous awards back at school and in local science fairs and two even published their work (see accompanying abstract). The program is now funded by a grant from the NIEHS and will be expanded in 2010 due to an award under The American Recovery and Reinvestment Act of 2009.

Graduate students interested in working with the students should contact Professor Field and attend organization sessions held over the winter. High school students should apply through the website at: <http://www.med.upenn.edu/ceet/summerprograms.shtml>





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[www.med.upenn.edu/ceet/](http://www.med.upenn.edu/ceet/)

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