Center of Excellence in Environmental Toxicology (CEET)

TWELFTH ANNUAL SYMPOSIUM

Windows of Susceptibility

Arthur H. Rubenstein Auditorium
Smilow Center for Translational Research
Perelman School of Medicine at the University of Pennsylvania

June 19, 2017

Perelman School of Medicine University of Pennsylvania | CEET
CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

NIH National Institute of Environmental Health Sciences

Contents

Schedule	1 – 2
Welcome	3
New in CEET	3
Keynote Speaker	4
Mission and Vision Statement	5
Members of CEET	6 – 7
Speakers	8 – 12
Poster Abstracts	
Lung and Airway Disease	13 – 17
Oxidative Stress and Oxidative Stress Injury	17
Reproduction, Endocrinology, and Development	18 – 20
Gene-Environment Interactions	21 – 22
Exposure Science	22 – 25
Community Outreach and Engagement Core	26 – 28
Translational Biomarker Core	29 – 31
Exposure Biology Infomatics Core	31
Superfund Research Program	32 – 37
Town Hall Meeting	38 – 40
Acknowledgements	42

Twelfth Annual CEET Symposium

Windows of Susceptibility

Monday, June 19, 2017

Smilow Center for Translational Research

7:30 – 8:30 A.M. REGISTRATION AND CONTINENTAL BREAKFAST

8:30 – 8:45 A.M. **Welcome & Opening Remarks**
Trevor M. Penning, PhD
Director, Center of Excellence in Environmental Toxicology

Jonathan A. Epstein, M.D.
Executive Vice Dean and Chief Scientific Officer
William Wikoff Smith Professor of Medicine
Perelman School of Medicine at the University of Pennsylvania

8:45 – 9:45 A.M. *IN UTERO* EXPOSURES
Moderator: George Gerton, PhD,
Director, Reproduction, Endocrinology, and Development Affinity Group

Topic 1: **Developmental Origins of Adult Disease: Is it Over Before Birth?**
Rebecca Simmons, MD
Professor, Pediatrics and CEET Deputy Director

Topic 2: ***In utero* Mouse Model of BPA Exposure: Physiology, Behavior and Epigenetics**
Marisa Bartolomei, PhD
Professor, Cell and Developmental Biology, University of Pennsylvania

Topic 3: **Human *in utero* Exposures to Endocrine Disrupting Chemicals**
Sara Pinney, MD, MS
Assistant Professor, Pediatrics, Children's Hospital of Philadelphia

9:45 – 10:45 A.M. POSTER SESSION WITH COFFEE

10:45A.M. – 12:15 P.M. POINT AND COUNTER POINT – ENDOCRINE DISRUPTING CHEMICALS
Moderator: Rebecca Simmons, MD, Deputy Director CEET

Topic 1: **Toxicology Findings on Bisphenol A (BPA)**
Luísa Camacho, PhD
Senior Staff Fellow, National Center for Toxicological Research,
Food and Drug Administration

Topic 2: **Environmental Epigenetics: A Mechanistic Link to Health, Disease and Intervention**
Shuk-Mei Ho, PhD
Jacob G. Schmidlapp Professor and Chair of Environmental Health
Director, Cincinnati Cancer Consortium
Director, Center for Environmental Genetics

Topic 3: **Perinatal Exposure of Rats to Low Doses of Zeranol Induces Transgeneration Effects on Sexual Development, Fecundity and Susceptibility to Mammary Carcinogenesis**
Helmut Zarbl, PhD
Professor of Toxicology, Department of Environmental and Occupational Health
Director, Center for Environmental Exposures and Disease,
Rutgers University School of Public Health
Rutgers Environmental and Occupational Health Sciences Institute

12:15 – 1:30 P.M. LUNCH

1:30 – 2:30 P.M. KEYNOTE
Environmental Hormones and Other Signals
John A. McLachlan, PhD
Celia Scott Weatherhead & Albert J. Weatherhead III Distinguished Chair in Environmental Studies
Professor, Department of Pharmacology, School of Medicine, Tulane University

2:30 – 3:30 P.M. POSTER SESSION WITH REFRESHMENTS

3:30 – 5:00 P.M. EXPOSURE SCIENCE
Moderator: Ian A. Blair, PhD
Director, Penn-SRP and Director, Translational Biomarker Core CEET

Topic 1: **Children's Health Exposure Analysis Resource (CHEAR)**
Lisa A. Peterson, PhD
Professor, Environmental Health Sciences, University of Minnesota

Topic 2: **Environmental Exposures and Neurodevelopment-Autism Spectrum Disorders**
Nathaniel Snyder, PhD, MPH
Assistant Professor, Drexel Autism Institute, Drexel University

Topic 3: **Challenges in Measuring Endogenous and Exogenous Hormones**
Clementina Mesaros, PhD
Research Assistant Professor, and Technical Director, Translational Biomarker Core of CEET

5:00 – 6:30 P.M. RECEPTION

Post Symposium - Town Hall Meeting
(Monday evening)

Chester Environmental Partnership, Chester, PA – an Environmental Justice Community
Sponsored by Community Outreach & Engagement Core, CEET

Host: Reverend Horace Strand, Chairman of Chester Environmental Partnership

Invited Guest: Dr. Linda Birnbaum, Director NIEHS and NTP

See page 38-40 for details

12TH ANNUAL SYMPOSIUM

It is with enormous pride that I welcome you to the Twelfth Annual Symposium of the Center of Excellence in Environmental Toxicology (CEET) – the University of Pennsylvania Environmental Health Sciences Core Center.

Every year we choose a theme to embrace so that we can learn more about a field and how it might align with current and future directions of the CEET. This year's theme is on windows of susceptibility, recognizing that there are windows in our life-span that make us particularly vulnerable to environmental exposures. We have brought together a series of external speakers with expertise in exposures to endocrine disrupting chemicals, their possible mode of action, and the challenges faced in their detection and measurement.

We are particularly pleased that John McLachlan, PhD, the Weatherhead Distinguished Professor of Environmental Studies & Professor of Pharmacology at Tulane University, is this year's keynote speaker.

We would also like to invite you to our first Town Hall Meeting in Chester, co-organized by the Community Outreach and Engagement Core of the CEET and by the Chester Environmental Partnership in Chester PA, an Environmental Justice Community. We are delighted that Linda Birnbaum, PhD, DABT, ATS, Director of the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program will be present to listen to citizen's concerns. Dr. Birnbaum advocates the position that you cannot work in environmental health without working with communities. I encourage you to attend to what should be a lively session.

– Trevor Penning
Director, CEET

New in CEET

The Center of Excellence in Environmental Toxicology (CEET) was elevated this year to a school-based center which will provide additional resources for the growth of its mission. We now have active recruitments ongoing in translational asthma research and environmental epidemiology.

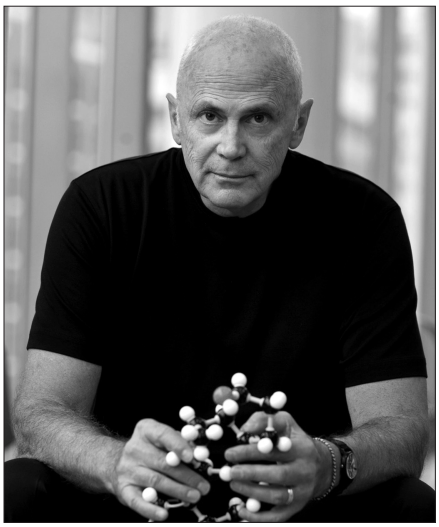
Reto Gieré, PhD

Chair, Department of Earth
and Environmental Sciences

We continue to build strong ties with the School of Arts and Sciences, especially with the Department of Earth and Environmental Sciences (EES). The Chair of EES, Professor Reto Gieré, now leads our new affinity group in Exposure Science with strengths in cartographic mapping, the use of GIS tools, air and water monitoring, biosensors and biowearables, and biomarkers.

CEET is also moving forward with EES to develop the first graduate degree program at Penn in environmental health sciences (EHS). The MS-EHS which will provide cross training in environmental science and environmental health sciences so that graduates will be able to protect the environment and the public from exposures that cause harm.

Keynote Speaker



John A. McLachlan, PhD
Celia Scott Weatherhead and Albert J. Weatherhead III
Distinguished Chair in Environmental Studies
Professor, Department of Pharmacology,
School of Medicine, Tulane University

John A. McLachlan received his undergraduate degree from the Johns Hopkins University where he was co-captain of the varsity football team. He is currently the Celia Scott Weatherhead and Albert J. Weatherhead, III Distinguished Chair in Environmental Studies, as well as holding a Professorship in the Department of Pharmacology in the School of Medicine, and an adjunct Professorship in Ecology and Evolutionary Biology in the School of Science and Engineering at Tulane University. From 1995 to 2012, he was also the Director of the Tulane/Xavier Center for Bioenvironmental Research (CBR), a comprehensive center that deals with environment in an inclusive manner.

Prior to coming to Tulane, McLachlan was Scientific Director at the National Institute of Environmental Health Sciences, NIH. While at NIEHS, Professor McLachlan developed the conceptual framework thirty-five years ago for what is now called *Endocrine Disrupting Chemicals*. Dr. McLachlan has published over 220 peer-reviewed papers and sixty review articles dealing with the environment and the reproductive system and, in the process, helped introduce the concept of epigenetics to environmental research and thinking. He has been a leader in research and communication about the environment and women's reproductive health.

At Tulane, Professor McLachlan established a translational research program on women's health focusing on ovarian hormones and the environment. He expanded the vision in hormone biology to include evolutionary aspects of hormone action. His research and outreach team emphasized understudied diseases like uterine fibroids. McLachlan's commitment to "use-inspired research" led him to explore community-based issues that could be approached in trans-disciplinary ways, using the Mississippi River as an overarching metaphor for research and teaching. Faculty from the humanities, performing arts, natural sciences, social sciences came together around the ideas related to urban centers in river deltas. For a period of time, Tulane operated the only research vessel dedicated to river research. A highlight of this effort was, in collaboration with the author, John Barry, the planning and design of *RiverSphere*, a research and cultural center located on seven acres of riverfront in the center of New Orleans.

In September 2005, Professor McLachlan confronted the aftermath of Hurricane Katrina by establishing the NSF-funded *Katrina Environmental Research and Restoration Network* to coordinate research and restoration and, from 2009-2012, was co-principal investigator on a multi-disciplinary NSF grant, entitled, *The "New Normal": The Impact of Trauma on Urban Ecological and Social Diversity* which studies how cities and communities function in the context of their natural ecosystems gaining a better understanding of resilience, recovery, and sustainability. McLachlan's restoration efforts focused on the Lower Ninth Ward. He also took an active role in public education serving on the organizing committee for the New Orleans Charter High School in Science and Mathematics recently rated one of the top high schools in Louisiana.

McLachlan has had a career long commitment to diversity in science and with various partners has maintained funded programs to facilitate the entry of minority students into scientific research since 1995.

McLachlan's current research focuses on using the principles of hormone signaling to understand how factors as diverse as trauma, stress, heavy metals and environmental chemicals exert their adverse effects on human health. He emphasizes differentiating systems such as stem cells for his studies and thinking.

Mission and Vision Statement

The Center of Excellence in Environmental Toxicology (CEET) is a school-based center housed in the Perelman School of Medicine at the University of Pennsylvania. As the spectrum of environmental health science is broad, ranging from toxicology, chemistry, environmental science, environmental disease, epidemiology, public health, and policy, its more than 60 members come from 16 departments and six schools as well as Children's Hospital of Philadelphia. CEET is Penn's designated Environmental Health Sciences Core Center (EHSCC) funded by the National Institute of Environmental Health Sciences (NIEHS). It is one of only twenty such Centers in the nation; it is the only one in the Commonwealth of Pennsylvania, and the only one in US EPA Region III (PA, DE, MD, WV, VA and Washington, DC). As such it is a regional and national resource.

The CEET elucidates the mechanistic links between environmental exposures and human disease and translates its findings into action to improve the health of vulnerable individuals, and local, national and global communities.

The CEET mission is achieved by both its community-based research model and by its affinity group structure. The Community Outreach and Engagement Core identifies community-based environmental health problems that are then framed by our Integrative Health Sciences Core Facility into research questions that can be answered by CEET investigators. Findings are then translated back to the community using a "community-first communication model". Ongoing examples include the fate, transport, remediation, and adverse health effects of asbestos exposure in Ambler in southeast Pennsylvania (which is home to one of the largest industrial Superfund Asbestos hazardous waste sites in the country). This work is now funded as part of the Penn Superfund Research and Training Program. Another example is the effect of natural gas drilling operations in the Marcellus Shale on public health where citizens in NE Pennsylvania are concerned about the effects of air pollution and water contamination on their health. An emerging theme is precision public health in which community exposomes can be used to identify sub-populations most vulnerable to asthma, and genomics to predict disease susceptibility.

The CEET Affinity Group structure was developed to tackle environmental health issues that pervade the region. The Affinity Group in Lung and Airway Disease examines the relationship between air pollution (ozone, fine particulate matter, allergens, SO2, NO2 and CO emission and asbestos) and disease (asthma, lung cancer, COPD, fibrosis and mesothelioma), and is underpinned by the Affinity Group in Oxidative Stress and Oxidative Stress Injury. Its Affinity Group in Reproduction, Endocrinology, and Development was formed to tackle issues of prematurity but now examines the relationship between exposures and windows of susceptibility, and is underpinned by the Affinity Group in Gene-Environment Interactions which has a strong presence in epigenetics. A new Affinity Group in Exposure Science uses data gathering tools to collect exposomic data at the community and individual level. Affinity Group investigators work together on integrated research themes that transcend the Affinity Group structure.

The CEET enables its investigators to conduct biomarker work using its Translational Biomarker Core, which uses sophisticated liquid chromatography mass spectrometry methods to identify and develop assays of biomarkers of exposure and effect. CEET investigators have access to an Exposure Biology Informatics Core so that large siloed data bases in genomics, proteomics, metabolomics and exposomics can be merged as predictors of response and disease onset. The Integrated Health Sciences Facility Core (IHSFC) of the CEET provides assistance with a broad range of transdisciplinary services including study design, exposure biology laboratories, population science services and, access to biospecimens via a CEET biorepository.

The COEC works with six communities in Pennsylvania to empower them with new knowledge so that they are better informed to influence decision makers about public health policy. To improve the environmental health of these and similar affected communities, the CEET educates health care professionals (Residency Program in Occupational and Environmental Health, Nursing concentration in Occupational and Environmental Health, and Masters of Public Health Programs) to improve public health outcomes.

CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

Perelman School of Medicine at the University of Pennsylvania

ADMINISTRATIVE CORE
Director: Trevor Penning, PhD
Deputy Director: Rebecca Simmons, MD

Affinity Group I
LUNG AND AIRWAY DISEASE

Director: Michael Beers, M.D

Steve Albelda, MD
Andrea Apter, MD, MSc
Eric Brown, PhD
Jason Christie, MD, MSCE
Melpo Christofidou-Solomidou, PhD
Peter DeCarlo, PhD*
David Feldser, PhD
Dongeun (Dan) Huh, PhD
Wei-Ting Hwang, PhD
Marcelo Kazanietz, PhD
Vera Krymskaya, PhD
Edward Morrisey, PhD
Trevor Penning, PhD
Joseph Testa, MD*
Anil Vachani, MD, MSCE

Affinity Group II
OXIDATIVE STRESS AND
OXIDATIVE STRESS INJURY

Director: Ian Blair, PhD

Paul Axelsen, MD
Joseph Baur, PhD
Michael Beers, MD
Eric Brown, PhD
Brenda Casper, PhD
Jeffrey Field, PhD
Aron Fisher, MD
Garret FitzGerald, MD
Reto Gieré, PhD
Harry Ischiropoulos, PhD
Douglas Jerolmack, PhD
Kelly Jordan-Sciutto, PhD
Vladimir Muzykantov, MD, PhD
Trevor Penning, PhD
Rebecca Simmons, MD
Andrew Strasser, PhD
Douglas Wiebe, PhD

Affinity Group III
REPRODUCTION, ENDOCRINOLOGY,
AND DEVELOPMENT (READ)

Director: George Gerton, PhD

Marisa Bartolomei, PhD
Shelley Berger, PhD
Samantha Butts, MD, MSCE
Christos Coutifaris, MD, PhD
Ted Emmett, MD, MS
Struan Grant, PhD
Jianghong Liu, PhD, RN
Sarah Millar, PhD
Katherine Nathanson, MD
Sam Parry, MD
Trevor Penning, PhD
Sara Pinney, MD, MS
Rebecca Simmons, MD
Nate Snyder, PhD, MPH*
Virginia Stallings, MD
Sigrid Veasey, MD
Jeremy Wang, MD/PhD

Affinity Group IV
GENE-ENVIRONMENT INTERACTIONS

Director: Marisa Bartolomei, PhD

Shelley Berger, PhD
Ian Blair, PhD
Jinbo Chen, PhD
Youhai Chen, MD, PhD
Jason Christie, MD, MSCE
Benjamin Garcia, PhD
Struan Grant, PhD
Hakon Hakonarson, MD, PhD
Blanca Himes, PhD
Hongzhe Li, PhD
Sarah Millar, PhD
Jason Moore, PhD
Katherine Nathanson, MD
Jennifer Pinto-Martin, PhD, MPH
Trevor Penning, PhD
David Raizen, MD, PhD
Sarah Tishkoff, PhD
Aalim Weljie, PhD

*Adjunct Member

CENTER OF EXCELLENCE IN ENVIRONMENTAL TOXICOLOGY

Perelman School of Medicine at the University of Pennsylvania

Affinity Group V

EXPOSURE SCIENCE

Director: Reto Gieré, PhD

Ian Blair, PhD
Charles Johnson, PhD
Peter DeCarlo, PhD*
Ted Emmett, MD
Krista Heinlen (consultant)
Amy Hillier, PhD (consultant)
Blanca Himes, PhD
Wei-Ting Hwang, PhD
Douglas Jerolmack, PhD
Jianghong Liu, PhD
Clementina Mesaros, PhD
Jason Moore, PhD
Trevor Penning, PhD
Kassashun Sellassie, PhD, PE*
Carsten Skarke, PhD
Nathaniel Snyder, PhD
Dana Tomlin, PhD (consultant)
Anil Vachani, MD
Douglas Wiebe, PhD

EXPOSURE BIOLOGY INFORMATICS CORE

Director: Jason Moore, PhD
Technical Director: Zhiping (Paul) Wang, PhD

TRANSLATIONAL BIOMARKER CORE

Director: Ian Blair, PhD
Technical Director: Clementina Mesaros, PhD

INTEGRATED HEALTH SCIENCES FACILITY CORE

Director: Anil Vachani, MD, MSCE

Human Studies Design and Performance Services
Director: Anil Vachani, MD, MSCE

Population Exposure Services
Associate Director: Ted Emmett, MD, MS

Virtual Biorepositories
Associate Director: Anil Vachani, MD, MSCE

Biostatistics
Associate Director: Kathleen Propert, ScD
Genetics Statistician: Mingyao Li, PhD
Statistician: Wei-ting Hwang, PhD

COMMUNITY OUTREACH AND ENGAGEMENT CORE

Co-Director: Marilyn Howarth, MD
Co-Director: Richard Pepino, MS

Maria Andrews, MS
Andrea Apter, MD, MSc
Fran Barg, PhD
Pamela Dalton, PhD
Jeff Field, PhD
Ira Harkavy, PhD
Michael Z. Levy, PhD
Jianghong Liu, PhD, RN
Judith McKenzie, MD, MPH
Kevin Osterhoudt, MD, MSCE
Pouné Saberi, MD, MPH

Marisa Bartolomei, PhD

Professor, Cell and Developmental Biology, University of Pennsylvania

Marisa Bartolomei is a Professor of Cell & Developmental Biology and co-Director of the Epigenetics Program at the University of Pennsylvania Perelman School of Medicine. Marisa S. Bartolomei received her BS in Biochemistry at the University of Maryland and then obtained her PhD from the Johns Hopkins University School of Medicine under the guidance of Dr. Jeffry Corden. She trained as a postdoctoral fellow with Dr. Shirley Tilghman at Princeton University. In 1993, Dr. Bartolomei was appointed as an Assistant Professor at the University of Pennsylvania and was promoted to Associate Professor with tenure in 1999 and Professor in 2006. In 2006, Dr. Bartolomei received the Society for Women's Health Research Medtronics Prize for Contributions to Women's Health. In 2011, Dr. Bartolomei received the Jane Glick Graduate School Teaching Award for the University of Pennsylvania School of Medicine and a MERIT award. She was elected as a Fellow of the American Association for the Advancement of Science in 2014 and is recipient of the 2017 Genetics Society Medal from the UK Genetics Society.

Luísa Camacho, PhD

Senior Staff Fellow, National Center for Toxicological Research, Food and Drug Administration

Dr. Luísa Camacho is a Senior Staff Fellow at the US Food and Drug Administration's National Center for Toxicological Research (FDA/NCTR). She received her BSc in Applied Plant Biology and her PhD in Cell Biology from the University of Lisbon, Portugal. She worked as a research associate at the University of Durham, UK, and as an academic visitor at the University of Oxford, UK. Dr. Camacho joined FDA/NCTR in 2007, where she has served as principal or co-principal investigator on several studies focusing on the toxicity of endocrine disruptors, including the CLARITY-BPA (Consortium Linking Academic and Regulatory Insights on BPA Toxicity) program. She has authored/co-authored 25 peer-reviewed articles in international journals and three book chapters, and is a peer-reviewer for several journals in the area of toxicology.

Shuk-Mei Ho, PhD

Jacob G Schmidlapp Professor and Chair, Department of Environmental Health, University of Cincinnati, College of Medicine

Director of the Center for Environmental Genetics, Director of the Genomics and Microarray Laboratory, Hayden Family Endowed Chair for Cancer Research, Director, Cincinnati Cancer Center

Internationally recognized for her expertise in endocrine disruption and hormonal carcinogenesis of the prostate, breast, ovaries and endometrium; Dr. Ho's research extends to the developmental basis of disease susceptibility by applying epigenetics to epidemiological studies, addressing two important challenges of research in environmental exposure and human health – multiple exposures at various developmental stages and the trans-generational effects of exposure. Her findings on EDC exposure, including Bisphenol A, helped raise concerns on *in utero* exposure to EDC in food and drinking water.

Her latest research focuses on estrogen signaling in prostate cancer, where her team recently discovered a new tumor-suppressive non-genomic estrogen signaling that can be exploited as a novel therapeutic option for advanced prostate cancer patients. Understanding this pathway may also help alleviate drug resistance associated with traditional hormone therapy.

Dr. Ho is an active participant in the Endocrine Society, the Society of Toxicology, the American Association for Cancer Research, Prostate Cancer Research Program, the American Urologic Society and the Society for Basic Urologic Research (past president). She has served on scientific review and policy committees for the National Institutes of Health and the Department of Defense, and on the National Academy of Sciences Standing Committee on Use of Emerging Science for Environmental Health Decisions. In 2007, she was honored by the Ohio Senate 127th General Assembly and was only the second woman to receive the Women in Urology Award from the SBUR and the Society of Women in Urology. The Prostate Cancer Foundation awarded her the Mentor of Excellence Award in 2013, and in August 2014, she was profiled in *Endocrine-Related Cancer* as a nationally and internationally recognized leader in the field. During 2015, the University of Cincinnati recognized her contribution by nominating and awarding her the George Rieveschl Award for Distinguished Scientific Research, and the Lifetime Achievement in Research.

Clementina Mesaros, PhD

Research Assistant Professor, and Technical Director, Translational Biomarker Core of CEET

Clementina Mesaros has been the Technical Director of the Biomarker Core Facility in the Center of Excellence in Environmental Toxicology since 2008. She has developed assays for metabolites involved in cellular oxidative stress and flux analysis of metabolites within intermediate metabolic pathways using stable-isotope dilution liquid chromatography mass spectrometry (LC-MS). Clementina also pursues her own independent avenue of investigation, which is focused on biomarkers of oxidative stress including isoprostanes, 8-oxo-dGuo and mechanisms of bioactive lipid formation and signaling pathways related to environmental exposures. As opposed to simply assessing exposure based in direct quantification of the exposure itself, this approach attempts to make use of downstream consequences of exposures. This technique is likely to provide complimentary information in settings of exposed populations.

Lisa Peterson, PhD

Professor, University of Minnesota

Lisa Peterson received her BS in chemistry at Macalester College, St. Paul, MN. She earned her PhD in Pharmaceutical Chemistry at the University of California at San Francisco in the laboratory of Dr. Neal Castagnoli, Jr. After post-doctoral studies at Vanderbilt University in the laboratory of Dr. Fred Guengerich, she joined the Division of Chemical Carcinogenesis at the American Health Foundation in Valhalla, NY. In 1997, Lisa moved to the University of Minnesota where she is now a Professor in the Division of Environmental Health Sciences and the Masonic Cancer Center. Her research focuses on mechanisms by which chemicals initiate carcinogenesis. Currently, she is investigating interactions between tobacco smoke chemicals in established rodent tumor models. In addition, she is co-Principal Investigator of the Minnesota CHEAR Exposure Assessment Hub in the Children's Health Exposure Assessment Resource funded by the National Institute of Environmental Health Sciences. Lisa has been an active member of the Division of Chemical Toxicology, American Chemical Society since its inception, serving as chair of the Bylaws Committee (1997-1998), councilor (2002-2004) and chair (chair-elect, 2008; chair 2009-2010; immediate past chair, 2011-2012). She has also served as Treasurer for the International Society for the Study of Xenobiotics. She is currently Associate Editor of *Chemical Research in Toxicology*.

Sara E. Pinney, MD, MS

Assistant Professor of Pediatrics, Perelman School of Medicine at the University of Pennsylvania

Attending Physician, Division of Endocrinology and Diabetes, The Children's Hospital of Philadelphia

The principal focus of Sara Pinney's research is to determine the molecular mechanisms that link an adverse intrauterine milieu to the development of adult disease. She is currently investigating how *in utero* exposures to environmental toxicants contribute to the development of diabetes and obesity in the offspring. The Pinney lab has measured concentrations of the endocrine disruptor bisphenol A (BPA) in human amniotic fluid and found that amniotic fluid BPA concentrations are associated with decreased birth weight of the offspring. In addition, she correlates amniotic fluid BPA concentrations with genome wide DNA methylation patterns and gene expression profiles measured with RNA-Seq in corresponding amniocytes, a fetal derived cell with stem cell properties. These projects are examples of how her laboratory is taking a translational research approach to investigate the role of *in utero* exposure to environmental toxicants in the programming of diabetes and obesity in the offspring. Their long-term goal is to understand the molecular mechanisms by which environmental toxicants can affect the developing fetus and program the development of diseases, such as diabetes and obesity that may present later in life.

Rebecca Simmons, MD

Professor, Pediatrics Perelman School of Medicine at the University of Pennsyvania

Attending Physician, The Children's Hospital of Philadelphia

Deputy Director, CEET

Dr. Simmons completed her MD at the University of Arizona in Tucson and then went on to a Residency in Pediatrics at the University of Arizona Health Sciences Center followed by a Neonatal-Perinatal Medicine Fellowship at the Cardiovascular Research Institute in San Francisco, California. Dr. Rebecca Simmons is now the Hallam Hurt Endowed Chair and Professor of Pediatrics at the Perelman School of Medicine and an Attending Physician at the Children's Hospital of Philadelphia. Her research focuses on the causal mechanistic links between the intrauterine milieu and type II diabetes and obesity in the adult with a focus on epigenetics and mitochondria function. She and Marisa Bartolomei are collaborating on a project investigating the transgenerational effects of Bis-phenol A. Dr. Simmons is the Deputy Director of CEET and is the Co-PI of the March of Dimes Preterm Birth Research Center at the University of Pennsylvania.

Nathaniel Snyder, PhD, MPH

Assistant Professor, Drexel Autism Institute, Drexel University

Nathaniel Synder is an Assistant Professor at the A.J. Drexel Autism Institute, where he leads the Exposure Science Laboratory. His current work focuses on identifying and measuring modifiable risk factors for autism spectrum disorder (ASD). The long term goals of this work are to bridge population and individual level scientific approaches and develop a public health approach to prevention of ASD.

Nathaniel studied Biochemistry at the University of Maryland and trained at the National Institutes of Health. Dr. Snyder's Ph.D. thesis in Pharmacology at the University of Pennsylvania concerned analytical measurements of low abundance biological molecules using liquid chromatography-mass spectrometry (LC-MS). Also completed at the University of Pennsylvania, Dr. Snyder's MPH work investigated non-invasive biomarkers of asbestos exposure, and contributed to the Penn Superfund Research and Training Program. Nathaniel has published and presented academic works on analytical chemistry, metabolism, inflammation, and environmental exposure assessment.

Current research projects include studies of environmental exposures, metabolic pathways involved in neurodevelopment, and molecular mechanisms that may mediate ASD. Additional projects include fundamental work on improving sample collection and analysis, especially around gestational metabolism, as well as refining epidemiological trial design using laboratory measurements.

Helmut Zarbl, PhD

Professor of Toxicology, Department of Environmental and Occupational Health

Director, Center for Environmental Exposures and Disease,

Rutgers University School of Public Health

Rutgers Environmental and Occupational Health Sciences Institute

Dr. Helmut Zarbl has almost 35 years of research experience focused on understanding molecular mechanisms of toxicity, mutagenesis, carcinogenesis, toxicogenomics, as well as epigenetic and genetic mechanisms of disease susceptibility and chemoprevention. He received his Ph.D. in Biochemistry from McGill University in 1983, followed by a postdoctoral fellowship in the laboratory of Dr. Mariano Barbacid at the National Cancer Institute (NIH). He subsequently did a postdoctoral research with Dr. Paul Jolicoeur at the Clinical Research Institute of Montreal. He began his academic career at the Massachusetts Institute of Technology (MIT), in 1987, where he rose to the rank of Associate Professor and became the Deputy Director of their Environmental Health Sciences Center. In 1994, he joined the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle, WA, where he established, designed, staffed and directed the FHCRC's Genomics facility. He also served as the Director of Core Laboratories operated by the FHCRC Division of Public Health Sciences (PHS). He founded and served as the Director of the NIEHS Sponsored University of Washington/FHCRC Toxicogenomics Research Consortium, serving as the Director of the National Steering committee for two years.

In 2007 Dr. Zarbl joined the Department of Environmental and Occupational Medicine at Robert Wood Johnson Medical School at Rutgers, where he assumed the Directorship of the NIEHS funded Center for Environmental Exposures and Disease. He also served as the Associate Director at the Rutgers Cancer Institute of New Jersey from 2008-2013. He is currently Professor of Toxicology in the Department of Environmental and Occupational Health at the Rutgers School of Public Health. He served on national and international grant review panels, as well as scientific advisory boards to numerous university, non-profit and government agencies.

Dr. Zarbl is also the founding President of GeneAsses, Inc., a joint university and industry partnership whose mission is to translate research on differential susceptibility to environmental carcinogenesis into new diagnostic and prognostic tests, and therapies.

Lung and Airway Disease

L1 Identifying Barriers to Kras-mediated Lung Cancer Initiation and Progression

Michelle Cicchini[1,2], Elizabeth L. Buza[3], Kyra M. Sagal[1], A. Andrea Gudiel[1], Amy C. Durham[3], and David M. Feldser[1,2]

[1]Department of Cancer Biology, Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania; [2]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania; [3]Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania

Email: cicchini@upenn.edu

KRAS is the most frequently mutated proto-oncogene in lung adenocarcinoma. RAS signals through several pathways that have been implicated in oncogenesis, but amplification of the Mitogen Activated Protein Kinase (MAPK) signaling cascade is commonly associated with lung cancer progression. Recent work by our lab has shown that amplification of the MAPK pathway signaling leads to transformation of previously dormant oncogenic Kras expressing cells. Additionally, we demonstrated that amplification of the MAPK pathway was sufficient to drive adenoma progression to carcinoma. MAPK signaling and tumor progression are both affected by cell exogenous environmental signals such as growth factors, chronic inflammation, or chemical exposure. We hypothesize that environmental stimuli that induce MAPK signaling can promote the transformation of otherwise dormant $Kras^{G12D}$-expressing cells and contribute to lung adenocarcinoma progression. This project seeks to test the role of exogenous toxicants on MAPK signaling in Kras-driven mouse models lung cancer. The significance of this work will contribute to the understanding of whether malignant transformation in these models is mediated through environmental stimulants of MAPK signaling, which would could provide a viable therapeutic target for patients at risk for lung cancer development due to exposures to environmental toxicants.

Supported by T32ES019851 (M.C.), an American Association for Cancer Research-Bayer HealthCare Basic Cancer Research Fellowship (M.C.), grants from the National Cancer Institute R00-CA158581 and R01-CA193602 (D.M.F), and P30-CA016520 (Penn Abramson Cancer Center)

L2 Nrf2-Keap1 Signaling and Implications for the Metabolic Activation of Nitroarenes

Jessica R. Murray[1], Meng Huang[1], Clementina Mesaros[2], Volker M. Arlt[3], Karam El-Bayoumy[4], Albrecht Seidel[5], Ian Blair[1,2], and Trevor M. Penning[1]

[1]Center of Excellence in Environmental Toxicology, Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA, USA; [2]Center for Cancer Pharmacology, Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA, USA; [3]Analytical and Environmental Sciences Division, MRC-PHE Centre for Environment Excellence in Environmental Toxicology and Health, King's College London, London, United Kingdom; [4]Department of Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, Pennsylvania, USA; [5]Biochemical Institute for Environmental Carcinogens, Großhansdorf, Germany.

Email: murrayj@mail.med.upenn.edu

Diesel engine exhaust (DEE) is listed as a Group 1 Carcinogen by the International Agency for Research on Cancer and contributes to occupational and environmental causes of lung cancer. Nitrated-polycyclic aromatic hydrocarbons, or nitroarenes, are major constituents of DEE and are detected in ambient air pollution. Nitroarenes require metabolic activation to exert their mutagenic and tumorigenic effects. NQO1 is considered the primary nitroreductase in the metabolic activation of nitroarenes. However, AKR1C3 displays nitroreductase activity towards the cancer chemotherapeutic agent PR-104A and so we

Lung and Airway Disease

sought to determine whether AKR1C subfamily members contribute to toxification of nitroarenes. We have determined that AKR1C1-1C3 catalyze the nitroreduction of 3-nitrobenzanthrone, a representative nitroarene, using discontinuous UV-HPLC assays. Another representative nitroarene, 6-nitrochrysene, has the unique characteristic that it can be activated by both monooxygenation and nitroreduction. Here we demonstrate that AKR1C1-1C3 display dihydrodiol dehydrogenase and nitroreductase activity towards 6-nitrochrysene-1,2-dihydrodiol. The nitroreduction of diverse nitroarenes by AKR1C enzymes suggest that they may play a role in the activation of these diesel exhaust carcinogens. Notably both *NQO1* and the *AKR1C* genes are highly induced by Nrf2-Keap1-ARE signaling, suggesting that the antioxidant response may not be entirely protective in the context of DEE exposures. We have demonstrated that *AKR1C1-1C3* are highly upregulated by Nrf2 inducers R-sulforaphane and 1[2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole in immortalized human bronchial epithelial cells which raises the question of whether these potential chemopreventative agents may upregulate metabolic activation of nitroarenes. Bioreactive intermediates produced during the metabolic activation of nitroarenes can further upregulate expression of antioxidant response genes, indicating that nitroarenes may also induce their own metabolism via Nrf2-Keap1-ARE signaling.

Supported by P30-ES013508 to TMP and T32019851, PhRMA Foundation Pharmacology/Toxicology Pre-doctoral Fellowship to JRM

L3 Defining Sites of Instability Caused by ATR Inhibition and PAH Exposure

Nishita Shastri, Yu-Chen Tsai, and Eric Brown

Abramson Family Cancer Research Institute, Department of Cancer Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Email: nishita@mail.med.upenn.edu

Transient obstruction of DNA polymerase progression activates the ATR checkpoint kinase, which suppresses fork breakage, strand resection, and RPA accumulation. RPA ChIP-Seq has been used to identify 173 replication-perturbed locations (RPLs) across the mammalian genome from ATR inhibition. RPLs were typified by centrally-positioned simple repeats that have not been previously characterized, yet were enriched to a level that exceeded that of well-known difficult-to-replicate repeats. Structural and replication assays indicated that the purine-rich strands of these RPL repeats folded into unique intramolecular secondary structures and were capable of impeding DNA replication both *in vitro* and *in vivo*. To determine if replication stalling at these sites was sufficient to cause replication fork collapse into DNA double-strand breaks (DSBs) upon ATR inhibition, we developed a break-detection assay (BrITL) that demonstrated that RPL sites break. These studies are the first unbiased identification of replication sensitive-sites that rely on ATR for stability.

Polycyclic aromatic hydrocarbons (PAHs) are a class of more than 100 chemical compounds derived from a variety of sources, including fossil fuels, engine exhaust, and cigarette smoke. Once in cells, PAHs are oxidized into reactive diol epoxides (PAHDEs) that intercalate into DNA and form covalent linkages that promote mutations and double strand breaks (DSBs) during DNA replication. Although the point mutations produced by PAHs, as well as their targeting to some general sequence characteristics (e.g. G/C rich regions), has been documented, an unbiased genome-wide determination of where PAHs induce DSBs has not been reported. We propose that PAHs generate DSBs through persistent stalling of DNA replication forks, which culminates in their collapse into DSBs. Based on findings described above, we argue that these DSBs will depend not only on the presence of adducts at the site, but also on the chromatin and sequence context within which stalling occurs. To demonstrate this, we propose to adapt RPA ChIP-Seq and BrITL techniques to: 1) accurately identify the sites of benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) modification of DNA; 2) determine the location of replication fork

collapse and DSB generation from benzo[a]pyrene-trans-7,8-dihydrodiol(+/-) (BPDH) exposure; and 3) compare these sites to CNVs and breakpoints in lung cancers that have been linked to cigarette smoking.

Supported by a Pilot Project from 1P30 ES013508 from the National Institute of Environmental Health Sciences, NIH & by T32 ES019852 (Y-CT)

L4 Elucidating the Roles of Estrogenic Hormones in Mesothelioma Cancer Cells

Juliette A. Aka, Trevor M. Penning
Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

Email: julaka@upenn.edu

Malignant mesothelioma (MM) is a highly aggressive cancer with only about 10% patients surviving five years. The disease is associated with occupational and environmental exposure to carcinogenic minerals, majorly asbestos. Although epidemiological evidence indicates a higher incidence of MM in males due to occupational exposures, higher prevalence of MM in younger individuals (<55 years) and in women due to environmental exposure has been observed. Early exposure combined with a longer duration of exposure to asbestos may explain the high proportion of MM occurring in younger individuals; however, the causes of high occurrence of MM in women remain elusive. 17beta-estradiol (E2) is the most potent estrogenic hormone in women and mediates its effects by binding to estrogen receptors (ER-alpha and ER-beta) and to its membrane-bound receptor GPR30. Inverse correlations between the levels of E2 (in human MM samples) and mesothelioma cancer patient survival post diagnosis suggested decreased E2 levels inhibited mesothelioma cancer growth. Likewise, the aromatase inhibitor exemestane was found to inhibit the growth of MM cells. Nevertheless, treatment with E2 showed inhibition of MM cell proliferation via ER-beta (ERβ) *in vitro* and *in vivo.* These conflicting observations urge further study to explain the roles of estrogen in MM cells. Here we aim to investigate the role of ERβ and the estrogenic hormones estradiol and estrone on mesothelioma cancer cell growth and determine whether MM cells conduct denovo estrogen biosynthesis. Cells proliferation was measured after cell treatment with aromatase substrates (4-androstene-3,17-dione and testosterone) and inhibitors (letrozole, anastrozole and exemestane). Aromatase activity and cell hormone levels will be determined by stable isotope dilution liquid chromatography mass spectrometry (LC-MS). Using Western blot and RT-PCR, we showed that aromatase and ERβ expression were higher in REN MM cells rather than MSTO-211H MM cells. Our preliminary cell proliferation results showed that testosterone and aromatase inhibitors as well as the combination of both substrate and inhibitors modulated MM cell growth. The impact of ERβ on MM cell growth will be investigated using a selective ERβ antagonist, 5R,11*R*-cis-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (THC). This study will improve our understanding of the roles of estrogens and ERβ in mesothelioma, and will determine whether inhibiting estradiol synthesis or using adjuvant hormonal therapy has a role in the treatment of mesothelioma cancer in women. Our studies may also provide a mechanistic underpinning as to why women are susceptible to mesothelioma following environmental exposure.

Supported by P30-ES013508 and Discretionary Funding

Lung and Airway Disease

L5 Investigating the Role of Mutant p53 in Esophageal Squamous Cell Carcinoma

Ashley Lento[1], Apple Long[1], Veronique Giroux[1], Qiaosi Tang[1], Morgan Sammons[1], Andres Klein-Szanto[2], Shelly Berger[1], Anil Rustgi[1]

[1]Division of Gastroenterology, Department of Medicine, University of Pennsylvania, Philadelphia, PA USA; [2]Fox Chase Cancer Center, Philadelphia, PA, U.S.A.

Email: alento@upenn.edu

Esophageal squamous cell carcinoma (ESCC) is a highly aggressive cancer characterized by a high rate of metastasis, limited therapeutic options, and a poor prognosis. However, there is limited information regarding the molecular mechanisms underlying the metastatic properties of ESCC. p53 is one of the most commonly mutated genes in ESCC, and our group has shown that esophageal cells lines expressing a mutation in human p53 shows signs of malignancy and increased invasion in 3D organotypic culture. To elucidate the role of mutant p53 in ESCC we developed a novel mouse model utilizing a genetic and carcinogenic approach to recapitulate the genetic and environmental factors that play a role in human ESCC. 4NQO, a quinolone derivative, induces DNA damage similar to carcinogens present in tobacco. *L2cre;p53$^{-/-}$* and *p53R$^{172H/-}$* mice were generated and treated with 4NQO in their drinking water for 16 weeks, which resulted in the development of ESCC. Compared to wildtype mice, *p53R$^{172H/-}$* mice and *p53$^{-/-}$* mice exhibited a decreased tumor latency time, increased tumor frequency and a more severe tumor diagnosis. However, *p53R$^{172H/-}$* mice and *p53$^{-/-}$* mice displayed similar tumorigenic properties. The similar tumorigenic properties are likely due to additional mutations generated by the 4NQO insult. RNA-seq was performed on cell lines established from wildtype and p53 mouse models and reveled different gene expression profiles between wildtype, *p53R$^{172H/-}$*, and p53$^{-/-}$ cells. Pathway analysis revealed that the endocytic pathway is differentially regulated between p53R$^{172H/-}$ and p53$^{-/-}$ cells, and several endocytic recycling related genes, including Rab11-fip1, Rab25, and Myo5b are downregulated. Further examination of endocytic recycling can provide novel insight into the role of mutant p53 in ESCC tumorigenesis and lead to the identification of new therapeutic targets.

Supported by NIH P01-CA098101 to AKR, NIH/NIDDK P30-DK050306 Center of Molecular Studies in Digestive and Liver Disease to AKR, and 1F32CA206264 to AAL

L6 PKC Epsilon is a Mediator of KRAS-driven Lung Tumorigenesis

Rachana Garg[1], Michelle Cicchini[2], Martin Abba[3], David Feldser[2], Fernando Benavides[4], Steve Albelda[5] and Marcelo G. Kazanietz[1]

[1]Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; [2] Department of Cancer Biology, Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, PA, USA; [3]Centro de Investigaciones Inmunológicas Básicas y Aplicadas-CONICET, Universidad Nacional de La Plata, CP1900, La Plata, Argentina; [4]Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, TX 78957; and The University of Texas Graduate School of Biomedical Sciences at Houston, TX 77030, USA; [5]Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Email: rachanag@mail.med.upenn.edu

Environmental carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), are major causative agents of lung cancer, the leading cause of cancer related deaths in the United States. Oncogenic mutations in KRAS, one of the most common alterations found in non-small cell lung cancer (NSCLC), are induced in high frequency by PAHs and environmental carcinogens. Studies from many laboratories, including ours, established key roles for PKCε, a member of the protein kinase C (PKC) family, in mitogenesis and survival of cancer cells. Notably, PKCε is up-regulated in several epithelial cancers, including human

Lung and Airway Disease

NSCLC, suggesting a role in the development and/or maintenance of the malignant phenotype. We observed that PKCε inhibition/depletion blunt the proliferative, motile, and invasive properties of lung cancer cells with KRAS mutation (*PLoS One* 7:e31714, 2012). More importantly, silencing PKCε reduced the ability of human NSCLC cells with KRAS mutation to form tumors in nude mice as well as impaired their metastatic potential (*Oncogene* **31**:2593-2600, 2011). As Ras-transformed cells have enhanced DAG levels and signaling, we hypothesized that genetic targeting of PKCε reverses lung tumorigenesis driven by oncogenic Ras. To address this, we intercrossed lung-specific mutant Ras mice ($Kras^{LSL\text{-}G12D/+}$) with PKCε knockout mice. Notably, significant inhibition occurred in the formation of lung tumors upon loss of either one or both PKCε gene (*Prkce*) alleles, with significant extension of mice lifespan, suggesting a role for PKCε in the initiation of lung tumorigenesis driven by oncogenic mutant KRas. Furthermore, in silico database analysis of KRAS mutated human lung adenocarcinomas revealed a significant association between high PRKCE expression and poor patient outcome. As environmental lung carcinogens (PAHs) induce mutations in Ras, we next intended to test the hypothesis that PKCε is implicated in the action of these carcinogens. Interestingly, alveolar hyperplasia as well as pulmonary adenomas induced by benzo(a)pyrene, a prototypical PAH, were significantly reduced upon loss of one or two PKCε alleles, suggesting that genetic ablation of PKCε impairs chemically-induced lung carcinogenesis. Overall, our results indicate that PKCε is a novel effector of KRAS in lung cancer that may represent a promising target for disease treatment.

Supported by grant R01 to MGK, ES026023 from NIH.

Oxidative Stress and Oxidative Stress Injury

O1 **Mitochondrial DNA Adducts of Lipid Peroxidation Products with Rotenone**

Kevin Gillespie

Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

Email: gillespiekevinp@gmail.com

Environmental exposure to the pesticide rotenone is positively associated with Parkinson's disease incidence. Although rotenone-induced oxidative stress is well-documented, there is also evidence for mitochondria-selective injury in rotenone toxicity. Mitochondrial lipid peroxidation is one potential component of rotenone toxicity. ROS-induced lipid peroxidation generates electrophilic aldehydes, including 4-hydroxynonenal (HNE) and 4-oxononenal (ONE). These compounds generate nuclear DNA and protein adducts, but their mtDNA adducts are first explicitly reported here with a developed liquid chromatography tandem mass spectrometry method. This finding suggests that mitochondrial protein and mtDNA adducts of lipid peroxidation products may be biomarkers of selective oxidative injury of mitochondria in neurodegenerative disease.

Supported by T32 ES019851

Reproduction, Endocrinology, and Development (READ)

R1 Understanding the Role of the Aryl Hydrocarbon Receptor in the TCDD-Mediated Distortion of Embryo Sex Ratio in Male Mice

Kristin M Bircsak[1,2], Andrew M Prantner[1], Latresa Copes[1], George L Gerton[1,2]

[1]Center for Research on Reproduction and Women's Health and [2]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

Email: kbircsak@upenn.edu

Paternal exposure to the persistent organic pollutant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is associated with a skewed sex ratio towards a greater proportion of female offspring. This research aims to determine the effect of TCDD on embryo sex ratio, AHR localization in the testis, and the testicular expression of genes known to regulate sex ratio in mice. For the sex ratio experiments, WT C57BL/6 male mice were injected weekly with TCDD (wk 1: 2000 ng/kg, wks 2-21: 400 ng/kg) or corn oil vehicle and mated biweekly. Embryos were collected at gestation day 15.5 and genotyped for sex. For the AHR localization and gene expression experiments, mice were subjected to a similar treatment paradigm, yet for a shorter duration (3 wks). The sex ratio (number of males/total number of embryos) was significantly lower in embryos from TCDD-treated males relative to vehicle-treated males by 20% (Vehicle: 0.55 ± 0.02, TCDD: 0.45 ± 0.04), indicating that paternal TCDD exposure resulted in more female than male embryos. Furthermore, there was no difference in the percentage of Y chromosome-bearing sperm between treatment groups. Following 3 weeks of TCDD or vehicle exposure, AHR protein in the testis was similar between treatment groups and localized to the acrosomal region of round spermatids, the nucleus of elongating spermatids, and the cytoplasm of interstitial Leydig cells. Up-regulation of aryl hydrocarbon receptor (AHR) target genes, *Cyp1a1* and *Ahrr* in the liver and testis of males exposed to TCDD confirmed the activation of the AHR transcription factor-signaling pathway in both organs. Importantly, TCDD-treated males exhibited a 30% increase and a 20% decrease in the testis mRNA expression of genes involved in the determination of embryo genotypic sex, *Sycp3-like Y-linked* (*Sly*) and *Slx*, respectively. Thereby, *Slx:Sly* expression was reduced 40% compared to vehicle controls. While *Slx* was not detected in the liver, Sly gene expression was enhanced 40% in the livers of TCDD-treated mice. Taken together, these data confirm that paternal exposure to TCDD distorts embryo sex ratio and begins to shed light on a possible mechanism by which this phenomenon may occur.

Supported by R21ES024527, P30ES013508, and T32ES019851 (KMB)

R2 Early Life Exposure to BPA Results in Dose-, Generation-, and Sex-specific Adverse Physiological Outcomes in Adulthood

Frances Xin[1,2,3], Amita Bansal[1,4], Martha Susiarjo[1,5], Lauren M. Smith[6], Erin Fischer[1,2,3], Martha Stefaniak[1,2,3], Karl J. Jepsen[6], Rebecca A. Simmons[1,4], Marisa S. Bartolomei[1,2,3]

[1]Center of Excellence in Environmental Toxicology and [2]Epigenetics Institute and [3]Department of Cell and Developmental Biology, University of Pennsylvania, Philadelphia PA; [4]Department of Pediatrics, University of Pennsylvania and Children's Hospital of Philadelphia, Philadelphia, PA; [5]Department of Environmental Medicine, University of Rochester Medical Center, Rochester, NY; [6]Department of Orthopaedic Surgery, University of Michigan, Ann Arbor, MI

E-mail: francesx@mail.med.upenn.edu

Early life environment can impact disease risk later in life, a concept known as the developmental origins of health and disease (DOHAD). Our lab is investigating the effects of perinatal exposure to the ubiquitous endocrine disrupting compound bisphenol A (BPA) on multiple physiological endpoints in adulthood. Chronic, low-dose dietary exposure to BPA results in multiple adverse phenotypes. F1 and F2 generation male mice exhibit elevated body fat, glucose intolerance, and impaired insulin secretion. Depending on

the dose, the pancreas of F1 and F2 BPA-exposed male mice display reduced beta-cell mass or abnormal mitochondrial function in islets. In addition to altered metabolic health, F1 adult males exposed to BPA throughout gestation and lactation exhibit reduced bone strength as measured by four-point bending, while females are unaffected. Finally, behavior testing in F1 adult males demonstrates a more depressed-like state as measured by the forced swim test. Neither behavioral nor skeletal changes persisted into the F2 generation. Following the identification of functional changes in mice exposed to BPA, ongoing work is focused on identifying gross morphological as well as molecular changes in physiologically relevant tissues to identify mechanisms contributing to the observed phenotypes. Taken together, our physiologically relevant exposure paradigm has resulted in sex-, dose-, and generation-specific metabolic, skeletal, and cognitive health consequences.

Supported by P30-ES013508 to MSB, K99-ES022244 to MS, T32-ES019851 to FX, ES023284 and ES013508 to MSB and RAS

R3 Immune Cell Recruitment and Inflammation in the Pancreas Following Intrauterine Growth Restriction

Thea N. Golden[1], Lane J. Jaeckle Santos[1], G. Scott Worthen[2], Rebecca A. Simmons[1,2]

[1]Division of Neonatology, Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104; [2]The Children's Hospital of Philadelphia, Philadelphia, PA 19104

Email: goldent@mail.med.upenn.edu

Altered intrauterine milieu has the potential to increase risk of adult disease. Intrauterine growth restriction (IUGR) is a common pregnancy complication that restricts blood flow to the fetus thus causing an abnormal metabolic intrauterine environment. Common causes of IUGR are exposure to chemicals, like cigarette smoke, or maternal disease such as preeclampsia and malnutrition. Babies born small for gestational age (SGA) are more likely to develop Type 2 Diabetes (T2D) as adults. We use an experimental model of IUGR in which pregnant Sprague Dawley rats undergo surgery at gestational age 18 in which the uterine artery is ligated. This leads to pups born SGA that develop T2D as adults. Using this model, we tested the hypothesis that IUGR causes an inflammatory response in the pancreas. We used immunohistochemistry and ELISA to identify immune cells and analyze the inflammatory response in the pancreas at post natal day 14 (PD14). At PD14, serum leptin and insulin were increased. Immunohistochemistry identified macrophages (Cd68) and lymphocytes (Cd3) near and in the islets. Eosinophils (eosinophil peroxidase) were found primarily in the lymph nodes surrounding the pancreas and occasionally in the islet. Cells expressed TNFα in the exocrine tissue and inducible nitric oxide synthase (iNOS) and toll like receptor 4 (TLR4) in the islets and exocrine tissue. MCP-1 and RANTES were elevated in the serum and IL-2 in the islet. This work identifies immune cell infiltration and inflammation of the pancreas following IUGR. Through further identification of immune cells and phenotype, potential therapeutic targets may be identified.

Supported by R01 DK55704 (RAS)

Reproduction, Endocrinology, and Development (READ)

R4 Estrogenic Activity of Polycyclic Aromatic Hydrocarbon Ortho-quinones in Human Endometrium

Isabelle Lee, Clementina Mesaros, Trevor Penning

Center for Excellence in Environmental Toxicology and Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104-6061, USA

Email: islee@mail.med.upenn.edu

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous byproducts of incomplete combustion that are converted to reactive genotoxicants. In addition, they can act as ligands for the aryl hydrocarbon receptor (AhR), and some PAHs or their metabolites can activate estrogen receptors (ER), resulting in endocrine disruption. In one pathway of PAH activation, aldo-keto reductases (AKRs) convert PAH trans-dihydrodiols into PAH *ortho* (*o*)-quinones, which are then shuttled into the nucleus by AhR to modulate AhR-target gene expression. Given the similarity between planar PAH o-quinones and estrogen *o*-quinones, we hypothesize that PAH o-quinones can bind and activate ERs in estrogen target tissues e.g. endometrium. This activation may modulate ER-target genes leading to cell proliferation. We tested the estrogenicity of 3 PAH o-quinones (benzo [a] pyrene-7,8-dione (BPQ), benz[a]anthracene-3,4-dione and 5-methyl-chrysene-1,2-dione) in endometrial cells. We used the inducible alkaline phosphatase activity in Ishikawa cells, a human endometrial adenocarcinoma cell-line, as the read-out for ER activation. We demonstrated that these compounds induce ER activity, and that this activation is inhibited by Fulvestrant, an ER antagonist. We now demonstrate that the representative PAH, benzo [a] pyrene (BaP), and its metabolite, benzo [a] pyrene-7,8- dihydrodiol (BPD), upregulate AKR 1C1 and 1C3 expression, both at the mRNA and protein level which would be responsible for BPQ formation. Using high performance liquid chromatography and APCI mass spectrometry in the selected reaction monitoring mode, we find that BaP and BPD can be metabolized to the estrogenic BPQ in Ishikawa cells. Low micromolar concentrations of BPQ increase Ishikawa cell proliferation to the same level observed with nanomolar concentrations of estrogen, 17-α-ethinyl estradiol. Western blot analysis shows that Ishikawa cells express both ERα, and ERβ. Our work indicates that PAH *o*-quinones may play a role in the disruption of ER signaling in the endometrium most likely through ERα.

Supported by P30-ES013508 and T32-ES019851

Gene-Environment Interactions

GEI1 Investigating the Epigenetic Mechanisms Involved in Adverse Health Outcomes Following Prenatal DEHP Exposure

Tre Artis[1], Frances Xin[1,2,3], and Marisa Bartolomei[1,2,3]

[1]Department of Cell and Developmental Biology, [2]Center of Excellence in Environmental Toxicology, and [3]Center for Research on Reproduction and Women's Health, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Email: trartis@mail.med.upenn.edu

Environmental perturbations during fetal development may lead to molecular changes that increases one's risk of developing diseases in adulthood. Endocrine disrupting chemicals (EDCs) are ubiquitous in the environment due to their usage as plasticizers in many consumer products. One highly produced EDC is di-2-ethylhexyl phthalate (DEHP) and early life exposure to DEHP has been associated with adverse metabolic changes such as elevated body weight, body fat, and blood glucose. However, little is known about the role that epigenetic changes play in the establishment and transmission of these phenotypes. Thus, we hypothesized that one mechanism may be through stable changes in DNA methylation, a key epigenetic mark. To test this, we exposed F0 dams to one of several different doses of DEHP through their diet from pre-conception until either embryonic day 10.5 or weaning age (PND21). At PND21, offspring (and future generations) are no longer exposed and allowed to reach adulthood, at which time their metabolic health was assessed. Global DNA methylation levels were also assessed at both fetal and adult stages. Results show that F1 male offspring exposed to our highest dose (10 mg/kg bw/d) are significantly heavier by body weight than controls as early as PND21. However, such males only show moderate changes in blood glucose levels and body fat percentage. In addition, F1 males show significant loss of global DNA methylation in whole embryos and adult livers. These effects were sex specific and did not persist into the F2 generation. Future work will involve validation through an additional cohort and more tissue specific analysis focused on the liver, pancreas, and bone. Understanding the effects of prenatal DEHP exposure and the mechanisms through which it works will ultimately improve our knowledge of the risks it poses to human health.

Supported by RO1 ES023284

GEI2 Toward Understanding the Role of Behavioral Quiescence in *C. elegans* Following Ultraviolet Radiation Exposure

Kristen C. Davis[1,2], and David M. Raizen,[1,2]

[1]Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA, United States; [2]Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

Email: daviskr@mail.med.upenn.edu

Environmental exposures influence various parts of our lifestyles, including some aspects of which we are unaware. One significant portion of every person's life is sleep, whose biological function remains unknown. With so much time spent asleep, it is not difficult to imagine that exposures have an impact on the amount and quality of sleep. Exposures to various stimuli, that result in cellular stress, such as infection of ultraviolet radiation exposure, can promote sleep. To fully dissect the role of and genetic pathways involved in cellular stress-induced sleep, we employ the simple yet powerful genetic model organism *C. elegans*. In *C. elegans*, heat shock, viruses, ultraviolet radiation (UV), and other stressors that cause cellular stress, all result in enhanced sleep. We hypothesize that this sleep is induced to facilitate recovery from these stressors to repair the damage. This hypothesis predicts that the time course of recovery from cellular damage parallels the time course of recovery sleep after the injurious exposure, and animals that

Gene-Environment Interactions

are unable to sleep after the stressful exposure would be slower to recover from cell injury.

We are testing this hypothesis by measuring DNA damage at different time points following UV exposure. Double stranded DNA breaks that occur following UV radiation induce the phosphorylation of histone 2A variant X (H2A.X), which is recruited to the break to facilitate its repair. We find that pH2A.X+ nuclei are visible *in situ* immediately after UV exposure but are more clearly stained by 30 minutes after the UV radiation. We are also using immunoslot blots and antibodies to measure the induction and repair of the UV-induced (6-4) photoproducts and cyclobutane pyrimidine dimers.

By examining DNA damage repair over the course of sickness induced sleep, we are hoping to elucidate the role of sickness induced sleep following UV radiation.

Supported by R01-NS088432 to DMR and T32 ES019851 to KCD

Exposure Science

ES1 Biogenic Silica and Heavy-metals in Cyclone Ash from *Miscanthus Sinensis* – a Potential Risk?

Chiara Elmi[1], Patxi Garra[2,3], Alain Dieterlen[2], Gwenaëlle Trouvé[3], Volker Dietze[4], and Reto Gieré[1,5]*

[1]Department of Earth and Environmental Science, University of Pennsylvania, 240 S. 33rd Street, Philadelphia, PA, 19104-6313 USA; [2]Laboratoire MIPS, Université de Haute-Alsace, IUT de Mulhouse, 61 rue Albert Camus, 68093 Mulhouse, Cedex, France; [3]Laboratoire Gestion des Risques et Environnement, Institut Jean-Baptiste Donnet, Université de Haute-Alsace, 3 bis rue Alfred Werner, 68093 Mulhouse Cedex, France; [4]Air Quality Department, Research Center of Human Biometeorology, German Meteorological Service, Stefan-Meier-Str.4, 79104 Freiburg, Germany; [5]Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA, 19104 USA

Email: chiarael@sas.upenn.edu

Miscanthus is a promising non-food crop, yielding high-quality lingo-cellulosic material for both energy and fiber production. It is characterized by relatively high yields (regardless of rainfall, fertilizer or soil temperature), low moisture content at harvest, and high water- and nitrogen-use efficiencies. Since 1992, *Miscanthus* has been widely studied and is grown in Europe to produce biomass to burn for heat and electricity. Following the environmental regulations for air quality, most biomass thermal facilities include cyclone separators to capture fly ash contained in the flue gas. The aim of this study is to evaluate biogenic silica (phytoliths) content and heavy-metal concentrations in cyclone fly ash produced by the semi-industrial burning of *Miscanthus sinensis*. Environmental scanning electron miscroscopy (ESEM) revealed the presence of bilobate and trilobate phytoliths in *Miscanthus* straw, in samples from dry extraction, and in cyclone ash. Fluorescence microscopy observation of *Miscanthus* straw allowed for identification of shapes similar to those of *Miscanthus* epidermal phytoliths and bilobate phytoliths. ESEM-EDS point analysis of *Miscanthus sinensis* phytoliths selected from dry-extracted leaves revealed that the phytoliths were composed of Si and O. In the *Miscanthus sinensis* straw, Ca was found as an impurity in the Si-O phytoliths, but Ca was hosted by the surrounding leaf rather than the phytoliths. In the cyclone ash, the phytoliths additionally contained some K. X-ray power diffraction (XRPD) data document that the cyclone ash consists of nearly 83 wt% of amorphous material, which reflects the observed abundance of phytoliths in the *Miscanthus* straw and the dry extracts, as well as in the cyclone ash. The latter is characterized by high bulk SiO_2 concentrations (32 wt%). Our results document that the phytoliths are made of opal, $SiO_2 \bullet nH_2O$, which might pose a risk to workers handling the ash. The cyclone ash further contains a remarkable amount of heavy metals and metalloids, including Ag, Ba, Ce, Co, Cr, Cd, Cs, Sn, Sb, and radionuclides U and Th. Future studies will clarify the possible toxicological impact of biogenic silica and heavy metals in biomass and the derived combustion products.

Supported by P30 ES013508

Exposure Science

ES2 Urban Road Dust: A Geochemical and Public Health Assessment in Philadelphia, PA

Michael O'Shea[1], Chiara Elmi[1], Sarah Haber[1], and Reto Gierè[1,2]

[1]Department of Earth and Environmental Science and [2]Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA, United States

Email: michajo@sas.upenn.edu

Urban road dust is composed of particles accumulated on metropolitan roadways that can be derived from natural or anthropogenic sources. Frequent particle resuspension after settling leads to human interaction through a combination of inhalation, epidermal contact, and ingestion. Road dust can contain accumulations of heavy metals and other harmful substances, which makes it a potential urban environmental health hazard to those facing regular exposure. Children's higher rates of ingestion of the dust and their proximity to the ground greatly increase their exposures compared to adults. We sampled 30 sites within Philadelphia to test the variety of environments within the city and to determine the effect of different average daily traffic, truck traffic percentages, and geographies on the composition of road dust. We applied analytical techniques including X-ray diffraction (XRD), laser diffraction, inductively coupled plasma optical emission spectrometry (ICP-OES), and ashing to determine mineral phases present, particle size distribution, elemental composition, and organic content respectively. XRD results show five near ubiquitous mineral phases present: quartz (SiO_2), dolomite ($CaMg(CO_3)_2$), hematite (Fe_2O_3), anorthite ($CaAl_2Si_2O_8$) and magnetite (Fe_3O_4). Over 40 separate phases appear only once at various sample sites and in small quantities, suggesting that there is variability in the road dust. ICP-OES results suggest that sites near industrial areas, as well as those with high average daily traffic, have higher heavy metal concentrations. Also, the analysis indicates that lead is ubiquitous in road dust samples gathered and, at some sites, is present at concentrations above the EPA threshold for soil safety. Potential lead sources include lead-based paint from homes and buildings built before 1970, industrial sources, and lead combustion processes. The observed mineralogical and chemical variation in the studied samples can be due to the differences in surrounding geologic formations, soils, traffic, and construction materials, which all contribute to road dust. Urban road dust should be further explored as a potential contributor to the high risk of elevated blood lead levels associated with children living in Philadelphia.

Supported by P30 ES013508

ES3 Real-time Monitoring Bisphenol A using an Energy-efficient Sensing System Based-on Aptamer-Functionalized Graphene Transistors

Jinglei Ping and Charlie Johnson

Department of Physics and Astronomy, University of Pennsylvania, Philadelphia, PA, USA

Email: jping@sas.upenn.edu

Bisphenol A (BPA) is a toxic chemical with significant negative impact on brain, behavior, and prostate gland, especially in fetus, infants, and children. A major concern about BPA is the widespread human exposure from use and occurrence in the environment, making the detection of BPA particularly important in evaluating and reducing its perniciousness. Graphene, a two-dimensional carbon-based nanomaterial with high mobility and chemical inertness, is an extremely promising candidate for detecting BPA with high sensitivity. We report biosensors based on graphene field-effect transistors functionalized with BPA-selective aptamer. For effective atapmer-functionalization and BPA-testing, we developed a novel transistor-fabrication technique with graphene channel exposed on top and metal leads and gate buried underneath the graphene with an intermediate dielectric layer. We also designed a card-size signal pro-

Exposure Science

cessing system that can be programmed for signal conversion and data transmission. The integration of the ultra-low-power graphene-based biosensors and electronics enables automatic or point-of-use monitoring of BPA-level in environment for the lifetime of battery power supply.

Supported by CEET Pilot Grant 1P30 ES013508.

ES4 Evidence of Particle-phase Exposure to Third Hand Smoke in a Summertime Classroom

Anita M. Avery[1], Michael S. Waring[1], Peter F. DeCarlo[1,2]

[1]Department of Civil, Architectural, and Environmental Engineering, Drexel University, Philadelphia, PA, United States; and [2]Department of Chemistry, Drexel University, Philadelphia, PA, United States

Email: amavery@drexel.edu

Third hand smoke (THS) is an important exposure route of traditional and electronic cigarettes, as exposure can occur without the subject's knowledge. Nicotine, its oxidative products, and tobacco-specific nitros-amines (TSNAs) have been identified in the homes of smokers long after a smoking event because of their persistence in the indoor environment. This work measured indoor and outdoor aerosols simultaneously in a classroom at Drexel University in Philadelphia, using high-resolution time-of-flight aerosol mass spectrometry (HR-ToF-AMS). Positive Matrix Factorization (PMF) analysis of the organic aerosol fraction revealed three factors of outdoor- originated aerosol, and an indoor-originated factor comprised of reduced nitrogen organic aerosol (ROA). Signature peaks m/z 30 (CH_4N+), 42 (C_2H_4N+) and 58 (C_3H_8N+), are consistent with the smoking-associated amines and TSNAs. This is the first observation of bulk amine ROA in the aerosol phase with this instrumentation and PMF analysis. The ROA factor as THS was confirmed with follow-up experiments of sampling a jar of deposited smoke: the characteristic amine group fragments in the jar mass spectrum replicated that of the ambient ROA spectrum. Aerosol-phase partitioning of nicotine and related smoke products is limited by aqueous-phase aerosol availability. In this way, exposure to THS was variable to hygroscopic components of aerosol, humidity, and liquid water in the aerosol. As such, THS was not observed in dry wintertime sampling. In the summertime classroom, THS comprised nearly a quarter of the total particulate mass (PM) indoors, representing a new exposure route for THS. This is especially important for environments near smoking venues (entryways, stairways), and where recent smokers gather, including homes, classrooms, and meeting spaces.

Supported by NSF Award #1437916

ES5 Chemical Usage Patterns and the Production, Storage, and Disposal of Waste in the Marcellus Shale Region of The Commonwealth of Pennsylvania

Steven Vitale[1], George L. Gerton[1,2], and Trevor M. Penning[1,2]

[1]Center of Excellence in Environmental Toxicology and [2]Center for Research on Reproduction and Women's Health, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

Email: svitale@upenn.edu

In Pennsylvania's Marcellus Shale region, hydraulic fracturing ("fracking") is an unconventional gas (UG) extraction process that releases natural gas from deep shale that exists below approximately 75% of the Commonwealth's surface. Since 2007, the rapid growth of the hydraulic fracturing industry has positioned Pennsylvania as one of the major energy producers in the United States. The contributions to the business economy of the state are easily understood by acknowledging the profits accrued from the sale of

large quantities of natural gas. However, what is not fully appreciated at present is the full cost to society with regards to public health consequences. This study analyzes chemical usage patterns in Pennsylvania's hydraulic fracturing operations. Using publicly available toxicology and oil and gas production databases, we present a graphical overview of unconventional gas drilling activities involving known toxicants and disposal of solid and liquid waste that may impact public health. As part of our strategy we downloaded the FracFocus data-base which provides the chemical composition of hydraulic fracturing fluid for >35,000 wells which can be mapped to well location. This data-base identified more than 1,084 different chemicals used. These were then ranked in order of frequency of use from 2011-2015. Frequency-of use data were then used in heat maps in which chemicals were ranked by toxic end-point e.g. reproduction/developmental toxicity and genotoxicity, etc. We also have data from oil and gas production databases and have used geographical information systems approaches to map the production, storage, and disposal of waste from unconventional gas wells in Pennsylvania. Collectively, these studies provide geocoding of chemical exposures that can be used in subsequent epidemiological studies that associate chemical exposure with inpatient and outpatient hospitalization rates.

Supported in part by a pilot grant from CEET (NIEHS P30-ES013508)

ES6 **Assessing the Geospatial Distribution of Asthma Exacerbations in Philadelphia Using Electronic Health Record (EHR)-Derived Data**

Sherrie Xie, Rebecca Greenblatt, Michael Z. Levy, Andrea Apter, Michelle Ross, Blanca E. Himes

Department of Biostatstics, Epidemiology, and Informatics, University of Pennsylvania, Philadelphia, PA, USA

Email: xiex@vet.upenn.edu

Asthma exacerbations, episodes of worsening asthma symptoms requiring the use of systemic corticosteroids to prevent serious outcomes, are a major cause of morbidity and health care costs in the US. To effectively make progress toward understanding how demographic and environmental factors influence asthma exacerbations, Electronic Health Record (EHR)-derived data is valuable as a longitudinal repository of events affecting diverse populations. We obtained de-identified patient-level data for adult asthma encounters within the University of Pennsylvania Health System (UPHS) occurring between 2011 and 2014. Variables extracted included codified demographic information, geocodes corresponding to place of residence, ICD-9 encounter codes, prescribed medications, and patient addresses. We restricted our analyses to asthma patients who had at least one primary ICD-9 code for asthma and a prescription for albuterol. Cases were defined as patients who had at least one asthma exacerbation, defined as an encounter with a primary ICD-9 code for asthma and a new prescription for oral corticosteroids, while controls had no exacerbations. Spatial analysis was performed using generalized additive models with the *MapGAM* R package. From 2,748 cases and 5,464 controls, we determined through multivariate analysis that *black* race/ethnicity, older ages, grade ≥ 3 *obesity*, current or previous smoking history, and *Medicare* or *Medicaid* financial class vs. Private Insurance were independent predictors of asthma exacerbations. GAM analysis found that asthma exacerbations were associated with geographic location. The global test statistic against the null hypothesis that exacerbation odds did not depend on location was highly significant ($p<0.001$) suggesting that even after adjusting for covariates (i.e. race, age, BMI, smoking status, financial class), asthma exacerbations were highly spatially correlated. Local GAM tests identified hot spots with significantly increased exacerbation rates, including a region in Southwest Philadelphia ($p<0.01$). Our results suggest that EHR data is helpful to understand the geospatial distribution of asthma exacerbations in Philadelphia.

Supported in part by NHLBI R00 HL105663 (BEH), R01 HL133433 (BEH), T32 AI070077-08 (SX), and NIEHS P30 ES013508.

Community Outreach and Engagement Core

COEC1 Air Quality Outreach at Childcare Centers in Asthma Prevalent Philadelphia Neighborhoods

Thomas McKeon[1], Marilyn Howarth[1], Sean Greene[2], Frances Shofer[3]

[1]Center of Excellence in Environmental Toxicology and [3]Center for Public Health Initiatives and Department of Emergency Medicine, University of Pennsylvania, Philadelphia, PA, United States; [2]Air Quality Programs DVRPC, Philadelphia, PA, United States

Email: mckeont@mail.med.upenn.edu

Philadelphia has 2-3 times higher asthma rates than the State of Pennsylvania. Studies evaluating contributors to asthma have found outdoor pollutants to be important. The EPA provides daily air quality information at airnow.gov at which, anyone can sign up to receive free alerts. The purpose of this study was to determine whether childcare providers knew about poor air quality alert resources and would use them to benefit children in their care. The outreach program was designed to share information about asthma prevalence, dangers of poor air quality, and air quality alert resources with staff at childcare centers in Philadelphia. Prior to the outreach presentation, a survey was administered to evaluate staffers' baseline understanding. Post surveys were administered a month later to evaluate changes. Summary statistics were calculated and pre/post knowledge was compared using a paired t-test. 116 staffers attended the presentations. 87 completed both pre/post surveys. 92% of staffers surveyed never or rarely used air quality alerts before the program. Post survey, 40% of respondents reported signing up for air quality alerts and 70% used the information to benefit children in their care. Additionally, there was a 10.8% mean increase in knowledge score post survey (95% CI: 8.3-13.3%, p<.0001). This easily administered program using freely available information was shown to be of interest to childcare providers and increased their knowledge to benefit children in their care.

Supported by NIEHS Grant 5-P30ES013508-11

COEC2 Community Outreach: The Teen Research and Education in Environmental Science (TREES) Summer Program for High School Students

Jeffrey Field

Department of Department of Systems Pharmacology and Translational Therapeutics, Center of Excellence in Environmental Toxicology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia PA, 19104

Email: jfield@upenn.edu

In 2007, the Center of Excellence in Environmental Toxicology launched a community outreach education program for high school students called Teen Research and Education in Environmental Science (TREES) summer program. Graduate student mentors, returning high school student mentors, and faculty members volunteer their time to guide the students one-on-one and as a group. TREES includes daily lectures on environmental issues or "survival" skills, such as laboratory safety, library and internet research, ethics, writing, and presentation skills. TREES students also take part in two activities with undergraduates in our parallel STEER program. All attend a weekly "cutting edge" faculty lecture and a weekly field trip to and environmental site. TREES begins with two weeks of structured laboratory exercises to teach basic lab techniques. The basic training leads to the most unique aspect of the program: an individually guided research project on a topic chosen by the student. The students then present their individual research project in an oral symposium. TREES scholars have been highly successful in science fairs with most winning local awards, a number winning national honors, and several publishing their work. About 80% of the students major in STEM fields in college with about 25% majoring in environ-

mental science, far above the national average. A half-dozen alumni are now in graduate school with three pursuing graduate degrees in environmental sciences.

Supported by R25 ES016146 and R25 ES021649

COEC3 **Community Outreach: Short Term Educational Experiences for Research (STEER) Program for Undergraduate Students**

Jeffrey Field and Maria Andrews

Department of Department of Systems Pharmacology and Translational Therapeutics, Center of Excellence in Environmental Toxicology, University of Pennsylvania, Perelman School of Medicine

Email: jfield@upenn.edu

Each summer, the Center of Excellence in Environmental Toxicology runs a community outreach education program for undergraduate students interested in environmental health science called the Penn Undergraduate Environmental Health Scholars Program through the Short Term Educational Experiences for Research (STEER) grant. Approximately 8 students are accepted each year into this 10-week program. The heart of the program is working one-on-one with a faculty member on an environmental health research project. Each student is matched up with a faculty mentor, based on stated interests and career goals. The student-mentor relationship usually endures long after the program ends, with research often continuing into the school year. There is a weekly "Cutting Edge" lecture from faculty mentors. Past lecture topics have included endocrine disruptors, environmental justice, integrated pest management, natural toxins, and tobacco carcinogenesis. Other activities include a career panel and a discussion on responsible conduct of research. STEER also includes a weekly field trip to environmental sites. Past locations have included superfund sites, environmental justice communities, wastewater treatment facilities, wildlife refuges, and local gardens. Field trips and lectures take place with the TREES high school students (STEER scholars serves as role models for them). At the end of the program, the STEER scholars present their work in an oral symposium.

Supported by R25 ES016146 and R25 ES021649

COEC4 **High Throughput Platforms for Analysis of Fracking Water Samples**

Kevin Wang Yao Xiong and Jeffrey Field

Department of Department of Systems Pharmacology and Translational Therapeutics, Center of Excellence in Environmental Toxicology, University of Pennsylvania, Perelman School of Medicine, Philadelphia PA 19104

Email: jfield@upenn.edu

Hydraulic fracturing (fracking) is an unconventional gas (UG) extraction process that releases natural gas from deep shale. There is a particular concern about liquid waste because over four million gallons of water are used to frack each well, and the water is rendered non-potable afterwards. A recent CEET-sponsored study suggested there are health problems associated with fracking, including low birth weights and increased cardiac admissions, but the underlying causes or exposures were not identified. This is because of a lack of understanding of the nature of the exposures and mechanisms of actions of all of the toxicants present in the liquid waste. While the National Toxicology Program has characterized thousands of individual compounds, its focus on specific compounds may overlook the toxicology of complex mixtures. We are developing a high throughput toxicology platform to test water samples near fracking wells and to compare the samples to fracking wastewater. This is achieved through a series of specific reporter assays.

Community Outreach and Engagement Core

To date, we have tested and developed robust positive controls for the NFkappaB, Xenobiotic, NRF2 (ARE), HIF (hypoxia) and p53 (DNA damage) reporter systems. So far, our tests of the above reporters have failed to yield positive responses with fracking wastewater samples. Beyond the fracking water tests, the system can also analyze other emerging toxins, as well as determine the mechanism of action of unknown compounds and drugs. Additionally, we will also develop a sample collection network in the community. In the future, we will be able to develop toxicological profiles of water samples from the affected communities.

Supported by R25 ES021649 and CEET pilot funding.

COEC5 **My Community, My Air Quality: The GAMP Student Ambassador Program**

Jason Henderson, Jared Ruddick, Jessica Meeker, Richard V. Pepino

Center of Excellence in Environmental Toxicology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Poor outdoor air quality is associated with an increased prevalence of asthma and related air and lung pathologies. In 2012 the Public Health Management Corporation's Community Health Data Base estimated that 19.4% of adults in Philadelphia had asthma, a startling comparison to the 7.0% national prevalence. The South Philadelphia community in particular, faces a heavy burden of pollution from three major polluting sources: Philadelphia international airport, Philadelphia Energy Solutions refinery and I-95 and I-76, two major highways. In order to better understand the health risks this air toxics burden presents to the South Philadelphia community, CEET partnered with the local Girard Academic Music Program (GAMP) School to develop an air pollution module within the Environmental Science Program and to also develop new curriculum initiatives that focus on community based outreach to the school and local neighborhoods on air quality education.

In collaboration with US-EPA Region 3, the Philadelphia Air Management Services (AMS) provided funding to GAMP to develop an interactive curriculum that was integrated into this school's tenth grade physical science course and a high school Environmental Science elective course. With a focus on dissemination of public service information to peers and the community, students worked with CEET investigators on learning to translate air quality (AQ) data into a variety of public service announcements (PSA), and supporting presentations on asthma, the air quality index, and risks to sensitive populations. These homemade, student-driven PSA's were shared at an outdoor event at the school in June 2016 to educate community members on air quality and associated health risk information. This neighborhood and school collaboration event was an important step in closing the information gap between a community overburdened by air pollutants and regulatory agencies that often struggle to meet AQ standards. AMS was delighted with the initial investment in GAMP to engage the South Philadelphia community, and a round two of funding is currently being sought from EPA which would expand the program's boundaries to an adjacent Environmental Justice community that experiences many similar AQ issues.

Supported by P30 ES013508

Translational Biomarker Core

TBC1 Testicular versus Adrenal Sources of Hydroxy-Androgens in Prostate Cancer Patients during Neoadjuvant Androgen Deprivation Therapy

Tianzhu Zang[1,8], Mary-Ellen Taplin[2], Daniel Tamae[1,8], Wanling Xie[3], Clementina Mesaros[1,4,8], Zhenwei Zhang[3], Glenn Bubley[5], Bruce Montgomery[6], Steven P. Balk[5], Elahe A. Mostaghel[7], Ian A. Blair[1,4,8], Trevor M. Penning[1,4,8]*

[1]Department of Systems Pharmacology & Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States; [2]Harvard Medical School, Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, Boston, MA 02215, United States; [3]Harvard Medical School, Dana-Farber Cancer Institute, Boston, MA 02215, United States; [4]Center for Cancer Pharmacology, Department of Systems Pharmacology & Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, United States; [5]Beth Israel Deaconess Medical Center, Boston, MA 02115, United States; [6]University of Washington, Seattle Cancer Care Alliance, Seattle, WA 98109, United States; [7]Fred Hutchinson Cancer Research Center, Seattle, WA 98109, United States; [8]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania, PA 19104 United States

Email: zangt@mail.med.upenn.edu

Neoadjuvant androgen deprivation therapy (NADT) is one strategy for the treatment of early-stage prostate cancer; however, the long-term outcomes of NADT with radical prostatectomy including biochemical failure-free survival are not promising. One proposed mechanism is incomplete androgen ablation. In this study, we aimed to evaluate the efficiency of serum hydroxy-androgen suppression in patients with localized high-risk prostate cancer under intense NADT (leuprolide acetate plus abiraterone acetate and prednisone) and interrogate the primary sources of circulating hydroxy-androgens using our recently described stable isotope dilution liquid chromatography mass spectrometric method (Zang *et al.*, *J. Steroid Biochem. Mol. Biol*. 2017, **165**:342-355). For the first time, three androgen diols including 5-androstene-3β, 17β-diol (5-adiol), 5α-androstane-3α, 17β-diol (3α-adiol), 5α-androstane-3β, 17β-diol (3β-adiol), the glucuronide or sulfate conjugate of 5-adiol and 3α-adiol were measured and observed to be dramatically reduced after intense NADT. By comparing patients that took leuprolide acetate alone versus leuprolide acetate plus abiraterone acetate and prednisone we were able to distinguish the primary sources of these androgens and their conjugates as being of either testicular or adrenal in origin. We find that testosterone (T), 5α-dihydrotestosterone (DHT), 3α-adiol and 3β-adiol were predominately of testicular origin. By contrast, dehydroepiandrosterone (DHEA), 5-adiol, androsterone, epi-androsterone and their conjugates (either glucuronide or sulfate) were predominately of adrenal origin. Our findings also show that NADT failed to completely suppress DHEA-sulfate levels and that two unappreciated sources of intratumoral androgens that were not suppressed by leuprolide acetate alone were 5-adiol-sulfate and epi-androsterone-sulfate of adrenal origin.

Supported by a NCI Grant 1P01-CA163227-04 and P30-ES013508 awarded to TMP. The original COU-AA-201 study was supported by Janssen Research & Development.

Translational Biomarker Core

TBC2 Development of Urinary Biomarkers for Human Exposure to Petrogenic Polycyclic Aromatic Hydrocarbons (PAHs) Resulting from the Deepwater Horizon Oil Spill

Huang, M.[1], Mesaros, C.[1], Blair, I.A.[1], Croisant, S.[2], Elferink, C.[2], Penning, T.M.[1]

[1]Center of Excellence in Environmental Toxicology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, and [2]Center for Environmental Toxicology, University of Texas Medical Branch Galveston, TX

Email: mesaros@upenn.edu

The EHSCC at UTMB and Penn collaborate on the Gulf-Coast Health Alliance: health Risks related to the Macondo Spill (GC-HARMS). This consortium is conducting a human health assessment of gulf communities that may have been exposed to sea-food contaminated with petrogenic PAH. A critical component of this assessment is to identify and validate biomarkers of exposure. As a first step, the metabolism of nine representative petrogenic PAH (6-alkylated and 3-oxygenated) in human hepatoma (HepG2) and small intestine (CaCo2) cells was examined. Analytes were identified by RP-HPLC coupled with in-line fluorescence detection, ion-trap mass spectrometry (MS) and Obitrap HRMS to gain exact mass to 5 ppm. Common metabolites identified irrespective of the PAH examined included catechols and their conjugates, dihydrodiols, and evidence for bis-electrophiles which contained a diol-epoxide and o-quinone within the same structure. Urine from a convenience set of 10 gulf residents were compared to archival control urine samples from a non-exposed group for these metabolites. We found evidence for O-bis-methyl-retene -bis-catechol, 1-methyl-phenantherene-trans-dihydrodiol, and tetrahydroxy-5-methyl-chrysene-1,2- or 7,8-dione only in the gulf residents but not in the control samples. ROCs showed that these analytes had greater than 90% specificity and sensitivity to distinguish between the two groups. Further work will determine whether these are the first human biomarkers of oil exposure.

Supported by U19-ES020676; P30-ES013508; P30-ES006676

TBC3 LC-MS Determination and Pharmacokinetic Study of lonidamine in a Mouse Model of Melanoma

Lili Guo[1], Dominic Ciccimaro[1], Kavindra Nath[2], David S. Nelson[2], Jeffrey Roman[2], Clementina Mesaros[1], Jerry D. Glickson[2], Ian A. Blair[1]

[1]Penn SRP Center and Center of Excellence in Environmental Toxicology, Department of Systems Pharmacology and Translational Therapeutics, [2]Department of Radiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

Email: guolili@mail.med.upenn.edu

The anti-tumor drug lonidamine (LND; 1-(2,4-dichlorobenzyl)-1H-indazole-3-carboxylic acid) has been used in combination with other therapeutic agents to improve efficacy and overall response to cancer treatment. *In vitro* studies using cell culture models indicate LND significantly affects cancer cell metabolism by inhibiting monocarboxylate transporters, mitochondrial pyruvate carrier and mitochondrial electron transport chain. Despite the significant effect in suppressing tumor growth, LND was shown to cause acute hepatic toxicity in dogs receiving high dose of the drug. In order to understand the pharmacokinetics and biodistribution of LND in pre-clinical animal models, we developed a liquid chromatography tandem mass spectrometry method (LC-MS/MS) suitable for quantitation of LND and its metabolites in tissues and serum. A Thermo Quantum MS coupled with a Waters Acuity UPLC and the metabolites were monitored in multiple reactions monitoring (MRM) mode. Nude mice bearing human melanoma tumors were treated intravenously with LND at a dose of 100 mg/kg. Mice were sacrificed

Translational Biomarker Core

and serum, tumors, livers, kidneys, hearts and brains were harvested at different time points following the injection of LND. The method showed a linear range over three orders of magnitudes. The levels of LND varied vastly between organs. This method has paved the way for our future study to optimize LND delivery by nanoparticle and liposome as potential new approaches for its clinical application.

Supported by P42 ES023720, R01CA172820 and R01CA129544

Exposure Biology Informatics Core

EBIC1 **CEET Exposure Biology Informatics Core (EBIC)**

Zhiping Paul Wang[1,2], Steven Vitale[1,2], Trevor M. Penning[1], Jason H. Moore[2]

[1]Center of Excellence in Environmental Toxicology and [2]Institute for Biomedical Informatics, University of Pennsylvania, Philadelphia, PA, USA

Email: zhipwang@upenn.edu

The main goal of EBIC (http://upebic.org/) is to support CEET investigators with bioinformatics services for pilot projects and analysis of preliminary data for grant applications. We also assist investigators in the analysis of discrete data sets for manuscript publication. Project intensive bioinformatics support is available as a fee-for-service when investigators include EBIC in pending or awarded grants. Since its establishment in October 2015, EBIC has assisted CEET investigators on over 25 projects in the areas of NGS data analysis, multi-omics data management and integration, bioinformatics application development and machine learning. Here we present three active projects to showcase our capabilities.

To demonstrate our next-generation sequencing data analysis services, we present an automated and customizable RNAseq data analysis pipeline that was developed by EBIC. This suite enables raw data and alignment quality control and performs differential expression, gene ontology and gene-set enrichment analysis.

We show our work in scientific web-based applications through the Aldo-Keto Reductase (AKR) Superfamily database. This interactive site serves as a community repository for AKR genes, proteins, enzymes and potassium channels.

Lastly, we highlight our integration and design capabilities in the development of nanoscale sensors of environmental toxins. These embed aptamer-functionalized graphene field-effect transistors into microfluid channels to detect low levels of BPA, PCB, MDA, and heavy metals.

EBIC is pleased to consult on an individual basis to scope project need and support.

Supported by P30-ES013508 to TMP

Superfund Research Program

SF1 Absolute Quantification of Plasma Fibulin-3 as a Biomarker for Asbestos Exposure by Immunoprecipitation-high Resolution Mass Spectrometry

QingQing Wang, Liwei Weng, Clementina Mesaros, Ian A.Blair

Penn Superfund Research Program Center and Center of Excellence in Environmental Toxicology, University of Pennsylvania, Philadelphia, PA 19104

Email: qingqing@upenn.edu

Asbestos exposure is known to cause lung cancer and mesothelioma. The exceptionally long latency periods of most asbestos-related diseases have hampered its preventative treatment. New biomarkers are needed to detect asbestos exposure at an earlier stage and to individualize treatment. Fiblin-3 was recently reported as a new potential biomarker for pleural malignant mesothelioma. However, controversy results were reported due to unsatisfactory bioanalytical methodology or biological variability. In this study, we developed an immunoprecipitation approach coupled with nanoLC-high resolution mass spectrometry (NanoLC-HRMS) method for quantifying fibulin-3 in human plasma as a biomarker for asbestos exposure and evaluated its prognostic value.

Sheep Anti-fibulin-3 polyclonal antibody was selected out of five antibodies as it provides the highest specificity and pull-down efficiency. Dimethyl pimelimidate (DMP) was used to cross-link the antibody to protein A/G beads which enable fewer antibody contamination and less matrix effect on MS. Recombinant fibulin-3 was expressed from HEK293 cells using stable isotope labeling by amino acids in cell culture (SILAC) strategy, then it was spiked into plasma samples at initial step of sample preparation as an internal standard. Three signature peptides were selected from native fibulin-3 and SILAC-labeled fibulin-3. Parallel reaction monitoring (PRM) was used on Q Exactive HF (Thermo) for providing high selectivity and high sensitivity. Absolute quantification was based on the ratio of endogenous protein and SILAC-labeled protein. The low limit of quantification of current method reaches to attomole level in human plasma. This improved sensitivity and specificity was obtained by antibody-based immunoprecipitation step, as well as the use of HRMS under PRM mode. The enhanced sensitivity and specificity offered by the current method will allow for a more complete analysis of fibulin-3 and fibulin family, shedding light on previously unknown mechanisms of asbestos exposure.

Supported by NIEHS Grant: T32 ES019851 and P42ES023720

SF2 Accurate Quantification of Serum Protein Mesothelioma Biomarkers

Liwei Weng[1], Clementina Mesaros[1], Ian A. Blair[1,2,3]

[1]Centers of Excellence in Environmental Toxicology and [2]Penn SRP Center, [3]Department of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Email: wengl@mail.med.upenn.edu

High mobility group box-1 (HMGB1) is a non-histone chromosomal protein that is highly conserved in eukaryotic cells. It is known to play a regulatory role in inflammatory immune responses and has recently proved to be a potential novel therapeutic target in malignant mesothelioma (MM). HMGB1 normally locates in the nucleus. However, during cell necrosis due to asbestos fibers, HMGB1 undergoes acetylation followed by translocation from the nucleus to the cytoplasm, and then secreted to extracellular space, where it binds to and activates pro-inflammatory mediators. Given the role it plays in inflammatory processes, HMGB1 may hold promise as a biomarker of cell transformative processes and thus hold utility as an indicator of asbestos exposure. A recent report reveals that the serum levels of both HMGB1 and acetylated HMGB1 are elevated in MM patients using an HMBG1 ELISA kit. Herein, we developed a stable isotope dilution HPLC-MS method, which has higher sensitivity and specificity compared

with currently available HMBG1 ELISAs, to accurately quantify the HMGB1 levels and the acetylated HMGB1 levels in serum. Stable isotopically labeled HMGB1 was expressed using Stable isotope labeling by amino acids in cell culture (SILAC) strategy and was added as the internal standard (ISTD) at the initial step of the sample preparation. An anti-acetylation antibody was utilized to immunoprecipitate acetylated HMGB1 from the serum. In addition, dimethyl pimelimidate (DMP) was used to cross-link the antibody with the magnetic beads, in order to enable the elution of the target. Following elution, Glu-C digestion of HMBG1 yields peptides including two nucleus localization signal (NLS) fragments. These two key peptides are highly acetylated, which prevents HMGB1 from reentering the nucleus. Absolute quantification was achieved by analyzing the ratio of these two peptides from endogenous form and ISTD. The serum acetylated HMGB1 levels in mesothelioma patients were compared with healthy controls as well as with individuals that were heavily exposed to asbestos. The improved accuracy and enhanced sensitivity of this assay provides a thorough quantification method for acetylated HMGB1 and further identifies if the acetylated form is a promising biomarker for mesothelioma patients.

Supported by T32 019581 and P42 ES023720

SF3 **Stability of Asbestos Aggregates Subjected to Cycles of Drying and Wetting**

Ali Seiphoori and Douglas Jerolmack

Department of Earth and Environmental Science, University of Pennsylvania, Philadelphia, PA

Email: aliseiph@sas.upenn.edu

Well-dispersed asbestos fibers may move large distances in air, water and soil; however, under some conditions fibers may bind to each other to form large aggregates which hinder mobility. Natural soils experience cycles of wetting and drying from rain events; however, and it is not known how this wetting and drying influences the formation and stability of asbestos aggregates in the environment. We present here new laboratory experiments to evaluate the drying-induced aggregation of chrysotile asbestos suspension (well-dispersed fibers in the range of 1-30 μm length), followed by several cycles of wetting and drying. We extend our observations by conducting drying and wetting cycles on the aqueous suspension of silica microspheres (3.2 μm diameter). An inverted optical microscope and a high-speed/high-resolution camera were used to observe the air-drying process of the aqueous suspensions, while the subsequent wetting cycles were applied using an aerosol nebulizer for a minimum disturbance during the hydration process.

The results show that after the first drying process, the asbestos fibers form a pattern which is highly stable when subjected to subsequent wetting and drying cycles. A strong interparticle capillary interaction between asbestos fibers develop which largely reduce their mobility during next cycles. On the other hand, the aggregates of silica microspheres are observed to become easily resuspended during wetting, indicating a large mobility of silica particles. Interestingly, the presence of submicron silica ellipsoids along with the microspheres is observed to suppress the resuspension process during subsequent wetting.

These findings reveal the impact of anisotropic colloidal shape on the stability of drying-induced aggregates subjected to subsequent cycles of wetting and drying. This is an important aspect of colloidal transport in the natural environment where cycles of hydration and dehydration are expected due to a variation of the moisture content contributing to the offsite migration of contamination.

Supported by the National Institute of Environmental Health Sciences (NIEHS) through Superfund Grant P42ES 23720

Superfund Research Program

SF4 A Transparent Soil to Investigate the Transport of Asbestos in Porous Media

Ali Seiphoori, Carlos Ortiz, Douglas Jerolmacks

Department of Earth and Environmental Science, University of Pennsylvania, Philadelphia, PA

Email: aliseiph@sas.upenn.edu

Transport of asbestos through groundwater is typically considered to be negligible. Laboratory experiments suggest that the mobility of asbestos in soil may change due to surface chemistry interactions between two particles, but little is known about the mechanisms that govern mobility when many particles are involved. While the transport of colloids in porous media has been extensively studied theoretically, experimentally and computationally, few studies have examined the transport of highly elongated particles such as asbestos. By comparing asbestos particles with spherical particles, we hope to understand how particle shape influences the physical and chemical interactions of colloids with the soil substrate.

We present an experiment to investigate the aggregation and mobility of asbestos particles in porous media by developing an innovative flow-cell with an optically transparent (refractive-index matched with water) porous medium composed of granules of a fluoropolymer material. The water saturated porous medium enables study of chemical changes of pore water, while the surface potential of the granules as well as the pore size distribution of the medium is also tunable. This feature enables us to observe and track the particles within the porous medium using an optical microscope and a high-speed/high-resolution camera for a wide range of water chemistry and pore structure variations.

The aqueous suspension of chrysotile asbestos fibers was passed through this artificial soil substrate, while the pore-scale distributions of asbestos colloids were recorded. Beside the aggregation induced by anisotropic diffusion behavior, the range of asbestos particle size allows us to clearly demonstrate the size-dependent transport mechanisms including deposition, filtration, and retention of particles. To fundamentally understand the aggregate formation and environmental transport of colloids, aqueous suspension of silica microspheres and rods are also tested along with the asbestos fibers.

The findings from this research provide a link between asbestos colloids aggregation and transport processes at the interfacial, pore structure, and Darcy scales of the soil substrate, which helps to understand the fate and transport of asbestos fibers in the environment.

Supported by the National Institute of Environmental Health Sciences (NIEHS) through Superfund Grant P42ES 23720

SF5 The Synthetic Lignan Secoisolariciresinol Diglucoside (LGM2605) Prevents Asbestos-Induced NLRP3 Inflammasome Activation in Murine Macrophages

Ralph A. Pietrofesa[1], Patrick Woodruff[1], Wei-Ting Hwang[2], Priyal Patel[3], Shampa Chatterjee[3], Steven M. Albelda[1], Melpo Christofidou-Solomidou[1,]*

[1]Division of Pulmonary, Allergy, and Critical Care Medicine and the Department of Medicine, [2]Department of Biostatistics and Epidemiology, [3]Institute for Environmental Medicine, Department of Physiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, 19104, USA

Email: melpo@mail.med.upenn.edu

Background: The interaction of asbestos fibers with macrophages drives two key processes that are linked to malignancy: (1) the generation of reactive oxygen (ROS)/nitrogen (RNS) species and (2) the activation of an inflammation cascade that drives acute and chronic inflammation, with the NLRP3 inflammasome, IL-1ß and TNFα playing key roles. Synthetic secoisolariciresinol diglucoside (SDG), Secoisolariciresinol diglucoside (SDG) is a non-toxic, flaxseed-derived pluripotent compound that has anti-inflammatory and antioxidant properties and may potentially function as a chemopreventive agent. We evaluated the

effect of synthetic SDG (LGM2605) on conferring protection from asbestos by studying the effect of SDG-pretreatment on asbestos-exposed murine peritoneal macrophages (MF). Methods: MFs were exposed to crocidolite asbestos +/- LGM2605 given 4 hours prior to exposure and evaluated at various times for NLRP3 expression, secretion levels of inflammasome-activated cytokines (IL-1ß and IL-18), proinflammatory cytokines (IL-6, TNFα and HMGB1), NF-κB activation, and indices of cell injury markers malondialdehyde (MDA), and total nitrates/nitrites. Results: Asbestos induces a significant ($p<0.0001$) increase in the NLRP3 subunit, release of proinflammatory cytokines, NLRP3-activated cytokines, NF-κB and levels of nitrates/nitrites. LGM2605 significantly reduced NLRP3 ranging from 40-81%, IL-1ß by 89-96% and TNFα by 67-78%, as well as activated NF-κB by 48-49% while decreasing levels of nitrates/nitrites by 85-93%. Conclusions: LGM2605 reduced asbestos-induced NLRP3 inflammasome expression, proinflammatory cytokine release, NF-κB activation and nitrosative stress in murine peritoneal macrophages supporting its possible use in preventing the asbestos-induced inflammatory cascade leading to malignancy.

Supported by NIH-R01 CA133470 (MCS), NIH- 1R21AT008291, NIH-R03 CA180548, 1P42ES023720 and by pilot project support from 1P30 ES013508 awarded to MCS

SF6 **Tracing Androgen Metabolism with Inhibition of Aromatase in Breast Cancer: *In Vitro* Studies and Clinical Correlates**

Lisa Bottalico[1,2], Clementina Mesaros[1,2], Dominic Ciccimaro[1,2], Nathaniel Snyder[3], Angela DeMichele[4], Ian A. Blair[1,2]

[1]Superfund Research Program, NIEHS Center of Excellence in Environmental Toxicology and [2]Department of Systems Pharmacology and Translational Therapeutics, University of Pennsylvania, Philadelphia, PA; [3]Drexel University, Philadelphia, PA; [4]Rena Rowan Breast Cancer Center / Abramson Cancer Center, Perelman Center for Advanced Medicine, Philadelphia, PA

Email: bottlisa@mail.med.upenn.edu

Inhibition of aromatase in breast cancer has proven to be a remarkably effective therapeutic strategy to combat estrogen receptor (ER) positive breast cancers. The enzyme aromatase (CYP19A1) catalyzes the conversion of androgens to estrogens, and this conversion constitutes the primary source of estrogens in women after menopause. Aromatase inhibitors (AI) are a first line of therapy against ER-positive breast cancer, but acquired resistance is a significant problem. While the hallmark of acquired resistance is considered to be activation of growth signaling pathways capable of driving proliferation independent of ER, little is known about changes in estrogen or androgen metabolism that may occur locally to help the breast tumor adapt to growth in an estrogen-depleted environment. This study aims to investigate androgen metabolism and estrogen formation from androgen precursors in *in vitro* models of aromatase inhibitor therapy and resistance. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) methods developed in the Blair laboratory allow sensitive quantification of a panel of estrogen and androgen metabolites in human biological samples. This study represents an application of these methods to trace metabolism of 4-androstenedione and testosterone with and without inhibition of aromatase. Cell models include MCF-7Aro, an ER+ MCF-7 cell line in which aromatase is overexpressed and LTED-Aro, a derivative of MCF-7Aro which has adapted to growth in the absence of estradiol and is a model of late-stage endocrine therapy resistance. LC-MS studies demonstrate that among the third-generation AI, letrozole is consistently found to have the greatest potency for inhibiting aromatase *in vitro*. LTEDAro is observed to have enhanced aromatase activity beyond that seen in MCF-7Aro and alterations in metabolism of 4-androstenedione in the presence of AI can be observed in both cell lines. Current work is aimed at quantifying estrogens and androgens in serum of women on AI to trace individual therapeutic response.

Supported by NIH / NHLBI U54 HL117798, T32 GM008076 and NIH/NCI R25 CA101871

Superfund Research Program

SF7 Screening Plant for Phytoremediation of Asbestos Contaminated Site: What is the Best Choice?

Cedric Gonneau[1], Sanjay Mohanty[2], Jane Willenbring[2], Brenda Casper[1]

[1]Department of Biology, University of Pennsylvania, Philadelphia, PA 19104 USA; [2]Department of Earth and Environmental Science, University of Pennsylvania, Philadelphia, PA 19104 USA.

Email: cgonneau@sas.upenn.edu

Inhalation of asbestos fibers, a group of fibrous silicate minerals, can cause mesothelioma and other lung diseases. There are 1312 asbestos contaminated sites including Superfund, brownfield and naturally occurring asbestos sites in the US, and most of these sites are left untreated because current EPA-recommended remediation methods, which involve either moving or capping the contaminated soils are expensive. To minimize health risk from asbestos piles in these sites, a cost-effective and sustainable remediation strategy such as phytoremediation should be explored. In this study, we screened plant species for phytoremediation by conducting three complementary experiments in a greenhouse. First, eight commercial cultivar crops were grown in soil from a Superfund site (BoRit, PA) that has elevated concentrations of asbestos but low concentrations of heavy metals. Plant growth was compared to growth in a control medium (compost and sand). Then, two grasses species (*Andropogon. gerardii* and *Sorghastrum. nutans*) were used to test the effect of seed source (serpentine ecotype vs cultivar) and soil microbial inoculum (sterile and live) on the plant biomass in BoRit soil. Finally, two other species (*Sorghum bicolor* and *Brassica juncea*) were grown in sand and asbestos fiber mixture capped with one of five top soils, differening in organic carbon source.

Three crops species showed significant different growth among soils: *Brassica oleraceae, Andropon gerardii* and *Sorghum bicolor*. Biomass of the plants grown in BoRit soil was higher than biomass in control media. In each medium, biomass varied between species, and there was a species* seed source interaction. Biomass was higher for the *S. nutans* cultivar Holt than for the other *A. gerardii* cultivar and plants from serpentine ecotype. Serpentine soil inoculum had no effect on biomass. Addition of asbestos to sand caused a net loss of biomass without mortality. Top soil with higher amount of organic carbon enhanced biomass growth, especially for *S. bicolor*, but also caused asbestos fiber mobility in leachate. Thus, mobility of fibers in groundwater should be monitored in sites capped with organic-rich amendments. Overall, all experiments showed that crop cultivars can thrive in the presence of asbestos in soil, suggesting that phytoremediation can be a promising strategy for remediation of asbestos contaminated sites.

Supported by P42 ES02372

SF8 Characterization of Asbestos Associated Mortality in Ambler, PA

Michelle Ross

Department of Biostatistics and Epidemiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia PA 19104

Email: michross@mail.med.upenn.edu

Ambler, Pennsylvania is home to one of the largest asbestos waste sites in the US. Community and occupational exposure to asbestos began in the late 1800s and continued until the 1980s. We examined the influence of asbestos exposure on mortality using an historic cohort. The population consisted of 4,524 individuals enumerated in the 1930 census, where vital status information was obtained from ancestry.com. We compared survival curves by age, sex and race across different exposure groups. Among those less than 18 years of age, we found a significant difference in survival for girls residentially exposed to asbestos compared to girls not residentially exposed to asbestos, while this same phenomenon was not

observed for boys. This may be an indication of a biological difference in susceptibility to asbestos and warrants further investigation.

Supported by P42 ES02372

SF9 The Synthetic Lignan Secoisolariciresinol Diglucoside (LGM2605) Mitigates Libby Amphibole Asbestos-Induced immune Cell Activation in Mice and Murine Macrophages

Jean Pfau[2], Steven M. Albelda[1] and Melpo Christofidou-Solomidou[1]

[1]Division of Pulmonary, Allergy, and Critical Care Medicine and the Department of Medicine, Perelman School of Medicine, Philadelphia, Pennsylvania, 19104, USA and [2]Montana State University, Bozeman, MT 59718

Email: melpo@mail.med.upenn.edu

Background: Exposure to amphibole asbestos, such as the Libby Amphibole (LA) found in Libby Montana, has been associated with production of autoantibodies in mice and humans, and an increased risk of developing systemic autoimmune disease. Secoisolariciresinol diglucoside (SDG) a non-toxic, flaxseed-derived bioactive agent has diverse protective properties mainly due to its anti-inflammatory, anti-fibrotic and antioxidant properties. Our group identified potent protective properties from crocidolite-asbestos exposure modeled in mice. The current studies aimed to extend those findings by evaluating antioxidant and immunomodulatory effects of synthetic SDG (LGM2605) on asbestos-exposed mice and murine RAW264.7 macrophages (MF). Methods: Mice were given 100mg/Kg LGM2605 via gavage initiated 3 days prior to and continued for 3 days post a single i.p. dose of LA exposure (200 μg). Mice were evaluated on day 3 for immune cell influx in the peritoneal cavity using flow cytometry (FACS). Spleen WBC levels were also evaluated. MFs were exposed to LA asbestos +/- LGM2605 given 30 min prior or concurrently or 30 min post exposure to 10 μg/cm^2 LA and evaluated 24 hours later for cytokine secretion and levels of xCT transporter, a marker of oxidative stress. Results: In mice, LA induced a significant increase in spleen weight (p<0.0001), and increased peritoneal influx of lymphocytes, macrophages and granulocytes, both of which were significantly (p<0.0006) blunted by LGM2605. Importantly, LGM2605 significantly reduced B1a B cell levels in spleens, elevated by LA. The B1a B cells are key producers of autoantibodies and are implicated in autoimmune responses. Additionally, LGM2605 significantly (p<0.04) reduced TNFα secretion by MF as well as the expression of xCT when given at any time relative to the LA exposure. Conclusions: LGM2605 reduced LA asbestos-induced inflammatory cell influx, and WBC subtypes associated with autoimmune responses in mice. It also blunted proinflammatory cytokine release, and oxidative stress in murine macrophages supporting its possible use in mitigating the asbestos-associated diseases such as autoimmune disease.

Supported by NIH-R01 CA133470 (MCS), NIH- 1R21AT008291, NIH-R03 CA180548, 1P42ES023720 and by pilot project support from 1P30 ES013508 awarded to MCS

Chester Town Hall Meeting

Faith Temple Holy Church

1007 West 7th Street

Chester, PA

Chester Environmental Partnership, Chester, PA – an Environmental Justice Community

Purpose of Meeting
Marilyn Howarth, MD, FACOEM
Center of Excellence in Environmental Toxicology, University of Pennsylvania

Welcome
Rev. Horace Strand
Pastor, Faith Temple Holy Church
Chairman, Chester Environmental Partnership (CEP)

Reflections on Chester
John and Dolores Shelton
CEP members and residents of Chester

Comments
Linda Birnbaum, PhD, DABT, ATS
Director, National Institute of Environmental Health Sciences
and National Toxicology Program

Sponsored by Community Outreach & Engagement Core, CEET

The Chester Environmental Partnership was formed by Reverend Horace Strand in 2005, and its work has been key to many recent environmental improvements in Chester. In the early 1990s, Rev. Strand of the Faith Temple Holy Church founded Chester Residents Concerned for Quality Living (CRCQL) and became a major figure in the suit Chester Residents Concerned for Quality Living v. Seif, in which CRCQL argued that Pennsylvania's environmental agency permitting decisions had violated Title VI of the Civil Rights Act by allowing the clustering of environmentally unfriendly facilities in Chester. The case went to the US Supreme Court. Through such efforts it became clear that, although communities could make environmental gains through litigation, such gains were hard fought and often occurred long after the fact of the environmental harms.

Reverend Dr. Horace W. Strand, Sr.

Chairman, Chester Environmental Partnership

Dr. Horace W. Strand, Sr. attended the Chester-Upland School District until grade 11. He enlisted in the United States Marine Corps and graduated from the Kubasaki Far East Dependent School in Okinawa, Japan receiving an honorable discharge from the Marines. He enrolled and graduated from the Faith School of Theology in Charleston, Maine in 1978, where he graduated and received a Bachelor of Science Degree in Theological Studies .

In 1979, Dr. Strand founded the Faith Temple Holy Church and was awarded the honorary Degree of Doctor of Divinity at Jameson Christian College in Philadelphia, PA. In 1992, founder and first Chairman of Chester Residents Concerned for Quality Living (CRCQL) Dr. Strand addressed clustering of environmentally unsafe facilities within the community. CRCQL provided testimony to the National Environmental Justice Advisory Council (NEJAC), a federal multi-interest advisory group, concerning adverse environmental conditions existing in Chester. In December 1996, CRCQL filed a lawsuit against the PA Department of Environmental Protection alleging the Department's waste facilities permitting process violated Title VI of the Civil Rights Act of 1964 and EPA's implementing regulations because it disproportionately impacted the predominately African American residents of Chester. The case reached the U.S. Supreme Court and received national attention.

These actions were instrumental in the Commonwealth of PA's and EPA's environmental justice policy goals and actions. In 2005, Dr. Strand founded the Faith Temple Environmental Initiative, an outgrowth of CRCQL to address worsening conditions in Chester due to inaction on the part of agencies, regulations and the lack of community cohesiveness. Under the umbrella of Faith Temple Environmental Initiative and a grant from the Environmental Support Center, the Chester Environmental Partnership (CEP) was developed. The CEP hosted a leadership seminar for the purpose of educating and training Chester leadership about environmental health risks and promoting a cleaner healthier environment while attracting economic development and fostering jobs for the residents of Chester. The CEP consists of a coalition of local, state and federal government officials, academia and student representation, profit and non-profit organizations, churches and community leadership. The CEP addresses zoning, land use, permitting, environmental health and environmental health risk intervention, smart growth and partnerships to improve the quality of life and safety of the residents of Chester.

Among his many accomplishments, he received the NAACP George Raymond Freedom Award in 1995; the Environmental Community Service Award, presented by Wawa in 2009; the Pennsylvania Resources Council, Inc. Community Service Award in 2010; and the Martin Luther King, Jr. Drum Major For Justice Award in 2014.

Dr. Strand currently serves as Chairman of the Chester Environmental Partnership; a member of the Pennsylvania Department of Environmental Protection Environmental Justice Advisory Board; President, Delaware Sub-district; Member of Board of Presbytery, Northern District Convocation, Chairman of Northern District Nominating Committee; President, Chester Nehemiah Project; an active Community Development Corporation in the city Chester; Advisor to the Lloyd Street Civic Association, Chester, PA; Member of the Board of Directors of the Reach Alliance, Harrisburg, PA; and a Diamond Member of BAEO (Black Alliance for Educational Options). President of Children First America Delaware Co., (A PA EITC Scholarship Organization) and an appointee to the EPA National Environmental Justice Advisory Board (NEJAC).

Chester Town Hall Meeting

Linda Birnbaum,

Director, NIEHS and NTP

Linda S. Birnbaum, Ph.D., D.A.B.T., A.T.S., is the Director of the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH), and the National Toxicology Program (NTP). A board certified toxicologist, she has served as a federal scientist for over 37 years. Dr. Birnbaum is a former president of the Society of Toxicology, the largest professional organization of toxicologists in the world. She is the author of more than 800 peer-reviewed publications, book chapters, and reports, and is an adjunct professor at several universities, including Duke University and University of North Carolina. A native of New Jersey, Dr. Birnbaum received her M.S. and Ph.D. in microbiology from the University of Illinois at Urbana-Champaign. She is married and has three children.

ACKNOWLEDGEMENTS

The Center of Excellence in Environmental Toxicology would like to thank all those who made this Symposium possible including: Perelman School of Medicine at the University of Pennsylvania, the National Institute of Environmental Health Sciences, and the invited speakers.

http://ceet.upenn.edu

Design and artwork: Mary A. Leonard
Biomedical Art & Design, Perelman School of Medicine at the University of Pennsylvania