ALLEGHENY-ERIE REGIONAL CHAPTER
of the
SOCIETY OF TOXICOLOGY

ANNUAL REPORT: 2013-2014

May 1, 2013-April 30, 2014

I. Officers:

Office	2013-2014	2014-2015
President:	Aaron Erdely	Patti C. Erdely
President-Elect:	Patti C. Erdely	Phoebe Stapleton
Vice President:	Aaron Barchowsky	Aaron Barchowsky
Secretary:	Robin E. Gandley	Robin E. Gandley
Treasurer:	William J. Mackay	William J. Mackay
Past President	Timothy Nurkiewicz	Aaron Erdely
Councilors:	Mark Weisberg	Mark Weisberg
	Jim Scabilloni	Jim Scabilloni
	James P. Fabisiak	James P. Fabisiak
	Kelly A. Brant	Tim Nurkiewicz
	Elaine Freeman	Kelly A. Brant
	Hollie Skaggs	Elaine Freeman
PDA Representative:	Phoebe Stapleton	Kevin Beezhold
GSLC Representative:	Hannah Pope-Varsalona	Cody Nichols
K-12 Outreach:	Anne Simmons	William J. Mackay

President's comment: The last year was very successful for A-E SOT. This is directly attributable to the energy and commitment of the current officers. The conversion of having two smaller meetings per year to one single high impact meeting has been a tremendous success over the last couple years. A primary goal of A-E SOT is to continue this momentum and provide the region with an annual high impact meeting.

II. Committees:

Awards:	Jim Fabisiak
Communications:	Mark Weisberg
Education:	Aaron Barchowsky
Finance:	William Mackay
Membership:	Tim Nurkiewicz
Nominating:	Aaron Erdely
Program:	Patti Erdely, Aaron Erdely, Phoebe Stapleton
Web:	Jim Scabilloni

III. Activities:

a) 2014 A-E SOT Annual Meeting

Location / Date: Erickson Alumni Center, Morgantown, West Virginia. May 15th and 16th, 2014.
Registered Attendees: 74

Highlights:

1) Three Keynote speakers, Dr. Jack Harkema (top), Dr. Andrew Ghio (bottom left), and Dr. Alan Ducatman (bottom right) presented to begin each scientific session.

2) Two young investigators, three post-doctoral fellows and four graduate students, and one of our sponsors (RJ Lee Group) also presented in these symposia.

3) A conference dinner honoring Dr. Vincent Castranova was held at the Erickson Alumni Center on May 15th at 6:00 p.m. following the 4:00 p.m. poster and networking session. Fifty-five people attended the dinner, which featured a presentation by Dr. Castranova on the “Physical Chemical Properties of Particles which Drive Health Effects upon Inhalation.”

   As a token of appreciation for his charter membership in the regional Allegheny-Erie chapter, the chapter presented Dr. Castranova with a caricature drawn by Dr. Harkema. The caricature was framed, and the mat was signed by chapter members and dinner attendees.

4) 40 (including two late breaking) abstracts were presented during the two day meeting as part of the poster session or invited oral presentations.

5) "Lunch with an Expert" and the informal evening networking session with refreshments were both tremendous successes. Participation in the "Lunch with an Expert" session grew significantly from the previous year with a total of 16 graduate students and post-doctoral fellows attending. We received generous support from Qiagen/Ingenuity Systems for this activity. We were also grateful for the matching support from the SOT Career Resource and Development (CRAD) committee.

6) 2014 A-E SOT Awards:

2013 Maryanne Stock Student Research Award
Kylie Horvath; Mentor Dr. Thomas W. Simmons (pictured below)
Affiliation: Indiana Area Senior High School/Indiana University of Pennsylvania
Project: *Nitrite-Induced Methemoglobin Formation in Aquatic Larvae of the Midge*

2014 Graduate Student Travel Award
Cody E. Nichols
Dept. of Human Performance & Applied Exercise Science, West Virginia University
Mentor: Dr. John M. Hollander

2014 Postdoctoral Travel Award
Dr. Carol Starkey
Dept. of Biological Sciences, Marshall University
Mentor: Dr. Brian Antonsen

2014 Best Graduate Student Poster Presentation
Cody Nichols
Dept. of Human Performance & Applied Exercise Science, West Virginia University
Abstract: *Pulmonary Exposure to Carbon-Based Nanomaterials Induces Spatially-Distinct Cardia Mitochondrial Dysfunction*
Mentor: Dr. John M. Hollander

2014 Best Graduate Student Oral Presentation
Breanna Yingling-Farris
National Institute for Occupational Safety and Health and West Virginia University
Abstract: *Intratracheal Co-Exposure to Diesel Exhaust Particulate and Crystalline Silica in Rats Potentiates the Inflammatory Effects of Silica in the Lungs*

Mentor: Dr. Jenny Roberts

2014 Best Postdoctoral Fellow Poster Presentation
Dr. Tina Sager
National Institute for Occupational Safety and Health and University of Montana
Abstract: *Effects of Nanoparticle Pre-Exposure Dispersion Status on Bioactivity in the Mouse Lung*
Mentor: Dr. Andrij Holian and Dr. Vincent Castranova

2014 Best Postdoctoral Fellow Oral Presentation
Dr. Kevin Beezhold
Dept. of Environmental and Occupational Health, University of Pittsburgh
Abstract*: Maladaptive Signaling in Response to Arsenic Exposure Impairs Cardiac Bioenergetics and Enhances Autophagy*

2014 Best Methodology
Hannah Pope-Varsalona
Dept. of Environmental & Occupational Health, University of Pittsburgh
Abstract: *Tranlesion Synthesis Defends Against Telomere Dysfunction Induced by Hexavalent Chromium*
Mentor: Patricia L. Opresko

2014 Best Overall Poster
Dr. Elizabeth Engler-Chiurazzi
Center for Basic and Translational Stroke Research, West Virginia University
Abstract: *Behavioral Consequences of Prenatal Titanium Dioxide Nanoparticle Inhalation Exposure*

7) This past year A-E SOT was able to acquire funding from SOT to aid in student travel and lodging for our annual meeting attendance. The funding was beneficial and provided financial assistance to several students from area universities to attend the annual meeting.
8) The 2014 Mary Anne Stock Student Research Award has been determined, but not yet officially announced. Traditionally, this occurs after the deadline for our annual report.

*** ***For complete 2014 meeting details: see Attachment 1, Final Program. Additional photographs are available. We are happy to provide a CD with all photographs from our annual activities and meeting if requested.***

IV. Newsletter: In 2013 we discontinued our Newsletter and associated committee. All "news-worthy" communications and announcements are posted on our website, and distributed via ToXchange.

V. Website: With the support of SOT (Raul Suarez), we overhauled our website in October 2012, and this is now an ongoing process (or living document). We had several requests at the 2014 annual meeting to archive the keynote presentations on the website and we intend to accomplish this goal with speaker permission.

VI. 2014 Annual Meeting: We have tentatively selected May 2015 for next year's meeting. This will be held again at the WVU Erikson Alumni Center (http://alumni.wvu.edu/eac). The WVU Department of Physiology & Pharmacology and the WVU Center for Cardiovascular & Respiratory Center have already committed financial and administrative support. Similar to our well-sponsored meeting in 2014, we will secure ample funding to continue providing a high level meeting, and interactions with relevant sponsors. Furthermore, our goal is to increase both attendance and sponsorship funding with the ultimate goal of increasing the presence/impact of A-E SOT in our region, and adjacent regions.

VII. New Meetings/Activities:

- The Student Committee (Chaired by Hannah Pope-Varsalona) initiated a Student Interests Meeting. This new meeting will be held quarterly, and the location will alternate between sponsoring Institutions. To date, this meeting initiated a student membership drive and added a very successful "lunch with an expert" to our 2013 and 2014 meeting programs. The “lunch with an expert” paired interested student members one-on-one with a senior member of the chapter either from industry, government, or academia based on the student’s request.

- At the National SOT meeting in Phoenix, A-E SOT combined with the Michigan chapter to host a joint reception. Virtually all A-E SOT members in attendance at SOT attended the joint reception and the event was considered a success by both chapters.

VII. Budget/Bank Statement: Generous donations from sponsors (The WVU Department of Physiology & Pharmacology, the WVU Center for Cardiovascular & Respiratory Center, Myriad / RBM, Qiagen, Ingenuity Systems, RJ Lee Group, DMT, TSI Inc., ThermoFisher Scientific) and a significant increase in meeting attendance (as well as an increased registration fee) resulted in ~$26,161 (June financial statement) in the A-E SOT account at the time of this report. When all sponsors are accounted for and balances have been paid, the chapter will have increased their account by ~$3000 compared to 2013. Furthermore, we are now operating in the black for three consecutive years while simultaneously increasing the size and impact of our annual meeting, and introducing new Chapter activities and public outreach. Two sponsors have been secured and several others are already being pursued for the coming fiscal year. Also, we will pursue SOT support next year for our annual meeting as well. The

purpose of this will be three-fold: 1) maintain our awards for graduate students, post-doctoral fellows and young investigators, 2) continue to develop/increase our annual meeting towards a "Current Concepts in Toxicology" meeting, and 3) increase our outreach impacts.

VIII. Outreach: A-E SOT has long-standing support for the Pennsylvania Junior Academy of Science (PJAS) and will continue this support. Anne Simmons, our current K-12 outreach coordinator, gave several lectures and related laboratory units on environmental toxicology to 86 elementary education majors. The primary goal from this activity was to educate the elementary education majors to prepare them for teaching toxicology to elementary students. For 2013-2014, William Mackay will lead our K-12 outreach. Several new avenues have been discussed since the 2013 annual meeting and will be pursued. These ideas include involvement in an ongoing educational environmental sampling study in the region, connect with the past President of the West Virginia statewide science teachers association to develop contacts for outreach in West Virginia, and pursue avenues to bring undergraduates to our meeting for exposure and to present.

VIV. Other: Administrative changes were developed by the officers and proposed to the greater membership. All proposals were approved by majority vote via ToXchange. The following changes to AE -SOT have been adopted:

Annual meeting registration was raised ($80 full/associate member; $135 non-member; $40 member post-doctoral fellow or graduate student; $80 non-member post-doctoral fellow or graduate student; $150 late, on-site registration).

28th Annual Meeting of the Allegheny-Erie Society of Toxicology Regional Chapter

Erickson Alumni Center
West Virginia University
Morgantown, West Virginia
May 15-16, 2014


A-E
SOT

PRELIMINARY SCHEDULE

Thursday, May 15

9:00 - 11:00 Registration
10:30 Lunch

11:00 – 11:25 Announcements

SYMPOSIUM #1:

11:25 – 1:35 ***Xenobiotic Toxicant Exposures and Biological Outcomes***
- 11:25 Introduction/Symposium Overview (moderator – Timothy Nurkiewicz, Ph.D.)
- 11:30 Keynote Speaker (Jack Harkema, Ph.D.; Michigan State University)
- 12:30 Hannah Pope-Varsalona (graduate student)
- 12:50 Kevin Beezhold Ph.D. (post-doctoral fellow)
- 1:10 Elizabeth Engler-Chiurazzi Ph.D. (post-doctoral fellow)

1:35-1:45 – Break

SYMPOSIUM #2:

1:45 - 3:30 ***Pollutant Characteristics and Toxicity Assessments***
- 1:45 Introduction/Symposium Overview (moderator – James Antonini, Ph.D.)
- 1:50 Keynote Speaker (Andrew Ghio, M.D.; U.S. EPA)
- 2:50 Valerie Minarchick (graduate student)
- 3:10 Jennifer Sisler Ph.D. (post-doctoral fellow)
- 3:30 Stephen Valentine Ph.D. (young-investigator)

4:00 Poster Session – Networking – Refreshments

6:00 Conference Dinner

Friday, May 16

8:00 - 9:00 Registration
A-E SOT Business Meeting

SYMPOSIUM #3:

9:00 - 11:00 **Toxicologic Challenges/Population and Occupational Risks**
- 9:00 Introduction/Symposium Overview (moderator – Aaron Barchowsky, Ph.D.)
- 9:05 Keynote Speaker (Alan Ducatman, M.D.: WVU School of Public Health)
- 10:10 Breanne Yingling (graduate student)
- 10:30 Alexander Ufelle (graduate student)
- 10:50 Gary Casuccio (RJ Lee Group)
- 11:10 Jenny Roberts Ph.D. (young-investigator)

11:40 - 12:30 "Lunch with an Expert" & Networking

12:30 - 1:00 Awards

1:00 Closing Comments and Adjourn

SPEAKER BIOGRAPHIES

1) Jack Harkema, Ph.D.

University Distinguished Professor
Pathobiology and Diagnostic Investigations
Michigan State University
East Lansing, Michigan

Dr. Harkema is widely recognized for his work in inhalation toxicology, nasal toxicology, and the toxicologic pathology of the respiratory tract in laboratory animals. His work is designed to understand the cellular and molecular mechanisms involved in the pathogenesis of airway injury caused by the inhalation of airborne pollutants. More specifically, his laboratory is investigating the roles of inflammatory cells and their mediators in the pathogenesis of airway epithelial alterations (e.g., apoptosis, necrosis, hyperplasia, metaplasia) in both the upper and lower respiratory tract after exposure (and co-exposure) to various inhaled xenobiotic agents (e.g., particulate matter, ozone, endotoxin, mycotoxins). His research also focuses on identifying biological factors (e.g., genetics, age, pre-existing disease) responsible for increasing airway susceptibility to toxicant-induced injury. The long-range goal of these studies are to understand the basic mechanisms of airway cell injury and repair in order to better prevent or treat the adverse effects of airborne pollutants.

2) Andrew Ghio, M.D.

The National Health and Environmental Effects Research Laboratory
United States Environmental Protection Agency
Research Triangle Park, North Carolina

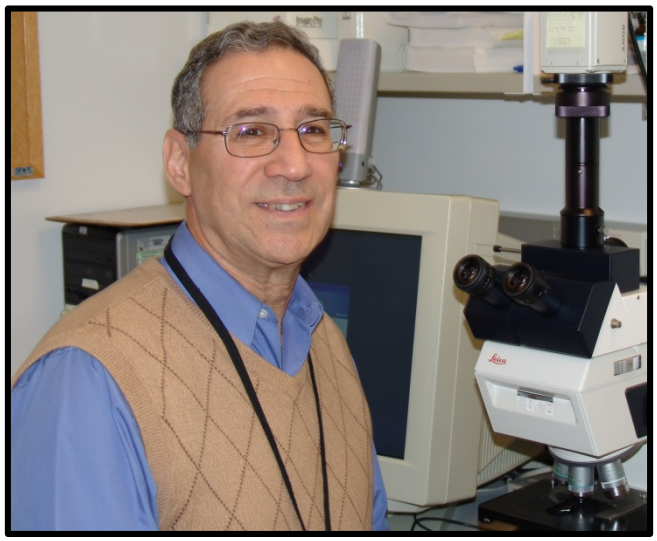
Dr. Ghio is a Medical Officer in the Clinical Research Branch of the Environmental Protection Agency. His research focuses on defining the response to particles and fibers including air pollution particles, silica, coal dust, and asbestos. This investigation has included cell biology and biochemistry into the role of iron and oxidative stress in human lung disease.

3) Alan Ducatman, M.D., M.Sc.

Professor
Department of Occupational and Environmental Health Sciences
West Virginia University School of Public Health
Morgantown, West Virginia

Dr. Ducatman's research interests include occupational and environmental toxicity and prevention of diseases potentially related to environmental exposures. His current environmental research focuses on the human population aspects of perfluorocarbon exposure. Similarly, Dr. Ducatman's clinic provides diagnostic and intervention advice to patients concerned with exposure to toxins. Dr. Ducatman also collaborates with clinical laboratory scientists and clinicians to create comparative effectiveness research for laboratory utilization practices. For both types of research, his aspiration is to improve the health of populations.

RECOGNITION OF DISTINGUISHED LEADERSHIP



Vincent Castranova, Ph.D.

Dr. Vincent Castranova is the Chief of the Pathology and Physiology Research Branch in the Health Effects Laboratory Division of the National Institute for Occupational Safety and Health, Morgantown, West Virginia. He holds the grade of a CDC Distinguished Consultant. He received the Shepard Lifetime Scientific Achievement Award from CDC in 2008, the Stokinger Outstanding Achievement in Industrial Toxicology Award from ACGIH in 2009, the ATS Assembly on Environmental & Occupational Health Val Vallyathan Senior Investigator Award in 2012, and the Inhalation and Respiratory Specialty Section/SOT Career Achievement Award in 2013. He is also an adjunct professor in the Department of Basic Pharmaceutical Sciences at West Virginia University, Morgantown, West Virginia and the Department of Environmental and Occupational Health at the University of Pittsburgh.

Dr. Castranova received a B.S. in biology from Mount Saint Mary's College, Emmitsburg, Maryland in 1970, graduating magna cum laude. He received a Ph.D. in physiology and biophysics in 1974 from West Virginia University, Morgantown, West Virginia before becoming an NIH fellow and research faculty member in the Department of Physiology at Yale University, New Haven, Connecticut. In 1977, Dr. Castranova received a research staff position at the National Institute for Occupational Safety and Health and an adjunct faculty position at West Virginia University, Morgantown, West Virginia. He has served at these institutions since that time.

Dr. Castranova's research interests have been concentrated in pulmonary toxicology and occupational lung disease. He has been coordinator of the Nanotoxicology Program in NIOSH since its inception in 2005. He has been a co-editor of four books, has given over 165 invited presentations, and has co-authored over 570 manuscripts and book chapters.

PRESENTATION ABSTRACTS

TRANSLESION SYNTHESIS DEFENDS AGAINST TELOMERE DYSFUNCTION INDUCED BY HEXAVALENT CHROMIUM

Hannah Pope-Varsalona, Fu-Jun Liu, Patricia L. Opresko

Department of Environmental & Occupational Health, University of Pittsburgh

Telomeres are repetitive nucleotide sequences that cap and protect chromosome ends. When telomeres become dysfunctional they contribute to a variety of pulmonary diseases. Our previous work established that DNA replication stress induced by the environmental pollutant hexavalent chromium Cr(VI) causes telomere loss and aberrations. Chronic inhalation of Cr(VI) leads to a variety of lung diseases, including fibrosis, and cancers. Cr(VI) forms a spectrum of DNA lesions that impede DNA replication and can cause collapse of the replication fork and chromosomal breakage. Telomeres are fragile DNA sites prone to breakage during replication stress. Cells have mechanisms for bypassing lesions that block replication forks called translesion synthesis (TLS). We hypothesize that Cr(VI)-induced DNA replication stress activates DNA Pol η which then suppresses Cr(VI)-induced telomere dysfunction. Our research is investigating several endpoints of telomere dysfunction in human cells proficient and deficient in polη. We observe that cells deficient in polη are 53 fold more sensitive to low levels of Cr(VI) and show through flow cytometry analysis that polη deficient cells are delayed in S-phase of the cell cycle compared to isogenic controls. Our data suggest that Cr(VI) induced delays in cell cycle progression is due to replication stress indicated by Cr(VI) concentration dependent increases of ATR foci. Cr(VI)-induced replication stress at telomeres is also shown by concentration dependent increases of ATR colocalized to telomere foci. Using a combination of immunofluorescence and telomere fluorescence in situ hybridization (IF-teloFISH), quantification of replication stress at genomic and telomeric DNA show that Cr(VI) induces polη foci formation at stalled DNA replication sites in human cells. Furthermore, we identify telomeric aberrations after Cr(VI) exposure by staining metaphase chromosomes with a fluorescent telomeric probe using teloFISH. We observe a four-fold increase in aberrations in cells with dysfunctional polη. Our study demonstrates one mechanism by which Cr(VI) directly interacts with the genome, alters telomere integrity, and the cellular pathways that protect telomeres in the face of genotoxic replication stress.

MALADAPTIVE SIGNALING IN RESPONSE TO ARSENIC EXPOSURE IMPAIRS CARDIAC BIOENERGETICS AND ENHANCES AUTOPHAGY

Kevin Beezhold, Linda R. Klei, Richard T. Cattley, Aaron Barchowsky.

Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health.

Environmental exposure to arsenic through drinking water causes cancers, as well as metabolic and cardiovascular diseases. Association of arsenic with cancer and many of its molecular mechanisms have been well studied, but much less is known of mechanisms for arsenic-promoted cardiac and vascular disorders. A low steady-state level of cardiac autophagy is critical to maintaining heart homeostasis. Increased autophagy in response to stress, such as ischemia or starvation are protective, while excessive response to reperfusion after ischemia is detrimental. Stress-induced autophagy, such as in arsenic exposure, can be adaptive and prevent cell transformation. We hypothesized that arsenic exposure increases maladaptive signaling in cardiac tissue causing impaired bioenergetics and enhanced autophagy. DNA binding arrays identified several dysregulated transcription factor families in arsenic-exposed mouse hearts. Pro-autophagic FoxO transcription factors were increased up to 6-fold above control along with Hif-1a. Protein analysis by immunostaining and Western showed increased expression of both FoxO1 and 3a in the heart nuclei and DNA binding for both Foxo1 and HIF-1a was confirmed by EMSA. Hearts from arsenic exposed (100mg/L in drinking water for 2-5 wks) mice were analyzed for FoxO- associated signaling pathways. Upstream of FoxO3a activation, we observed increased in miR-143, a cardiac and autophagy associated miRNA that increases FoxO3a protein expression. SRF, a transcription factor that drives miR-143 expression was increased and ELK1, a MAPK family member that is a direct miR-143 target decreased. Downstream of FoxO3a we observed increased pyruvate dehydrogenase kinase 4 (PDK4) expression that may mediate starvation signaling indicative of impaired bioenergetics. Additionally, p62 expression is decreased consistent with increased autophagy, and TEM suggests increased autophagy, mitophagy and mitochondrial dysfunction. These results suggest that arsenic increases autophagic flux in the heart by inducing autophagy regulating transcription factors (FoxO,HIF) and by eliciting maladaptive starved signaling (ELK1 and PDK4).

Supported by NIEHS F32 ES022134 (KB), and NIEHS R01 ES013781 (AB)

BEHAVIORAL CONSEQUENCES OF PRENATAL TITANIUM DIOXIDE NANOPARTICLE INHALATION EXPOSURE

Engler-Chiurazzi, EB[1], Stalnaker, JJ[1], Stapleton, PA[2], Nurkiewicz, TR[2], Simpkins, JW[1]

[1]Center for Basic and Translational Stroke Research, West Virginia University, Morgantown, WV, 26505

[2]Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV, 26505

Engineered nanomaterials, such as titanium dioxide (TiO_2), are commonly used in manufacturing and have a wide range of potential commercial applications. However, their toxic effects on the body and brain during the critical period for early nervous system development are unclear. Prenatal TiO_2 inhalation exposure results in alterations in uterine environment and birth outcomes (Stapleton et al., 2013). Further, prenatal TiO_2 via maternal injection accumulates in cranial nerves (Takeda et al., 2009), is associated with alterations in genes related to cell death, mitochondria, oxidative stress, and apoptosis, and increases dopamine in the prefrontal cortex and striatum of 3-6 week old offspring (Shimizu et al., 2009; Takahashi et al., 2010). Only one study has evaluated the effects of prenatal TiO_2 exposure on adult cognitive outcomes, finding that exposure was associated with decreased center time in the open field (a measure of increased anxiety) and altered pre-pulse inhibition (Hougaard et al., 2010).

The objective of this study was to determine if prenatal exposure to the nanomaterial TiO_2 affects adult brain and behavior. Pregnant rats were either unexposed or exposed to aerosolized TiO_2 5 hours/day for approximately eight days, with a final exposure of 11.3 +/-0.039mg/m^3. Male pups were utilized for the adult behavior battery. When the pups were five months old, we assessed performance on a battery of cognitive tests selected to tap into several different mnemonic functions, including the anxiety-like behavior (open field, elevated plus maze), spatial navigation memory (Morris water maze, radial arm water maze), locomotor coordination (rotarod), and depressive-like behavior (forced swim test). Detailed results of the cognitive behavioral battery evaluations will be presented. While we observed no impact of exposure to prenatal TiO_2 via maternal inhalation on measures of anxiety-like behavior, locomotor coordination, nor depressive-like behavior, we did observe an impact on spatial navigation memory. These findings suggest that prenatal exposure to TiO_2 can impart long-term effects on adult cognitive behaviors in male rats.

This research was supported by funding awarded to James W. Simpkins (NIA P01 AG0225500; NIA P01 AG027956), Phoebe A. Stapleton (PAS F32-ES02345), and Timothy R. Nurkiewicz (TRN R01-ES015022; TRN NSF-1003907).

DETERMINING A MECHANISTIC LINK BETWEEN CERIUM DIOXIDE NANOPARTICLES AND ARTERIOLAR DYSFUNCTION

VC Minarchick[1], PA Stapleton[1], NR Fix[2], SS Leonard[2], EM Sabolsky[3], TR Nurkiewicz[1]

[1]WVU Center for Cardiovascular and Respiratory Sciences, [2]National Institute for Occupational Safety and Health, [3]WVU Department of Mechanical Engineering, Morgantown, WV

Applications for cerium dioxide nanoparticles (CeO_2 NP) are potentially endless; however, its biologic interactions must first be understood. We previously reported that pulmonary CeO_2 NP exposure results in endothelium-dependent and -independent arteriolar dysfunction; however, the mechanism(s) of action is unclear. Furthermore, the variety of applications presents nonpulmonary exposure risks; however, the microvascular effects of other exposure routes are unknown. Based on previous observations, we hypothesized that arteriolar dysfunction is mechanistically linked to impaired nitric oxide (NO) signaling and this dysfunction will be dependent on exposure route.

Rats were intratracheally instilled (65 µg), intravenously injected (100 µg), or gavaged (400 µg) with CeO_2 NP suspended in saline. Control animals were exposed to saline. Mesenteric arterioles were examined 24 hours later via isolated vessels. Arteriolar reactivity was evaluated by using acetylcholine (ACh, 10^{-9}-10^{-4} M) and spermine NONOate (10^{-9}-10^{-4} M). The roles of NO synthase and cyclooxygenase were tested in the presence of N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NMMA, 10^{-4} M) or indomethacin (INDO, 10^{-5} M) (respectively). Soluble guanyl cyclase activator (YC-1) and cyclic guanosine monophosphate mimetic (8-Bromo-cGMP) assessed smooth muscle activation. Electron spin resonance and the Apollo 4000 free radical analyzer assessed the ability of NO to react with CeO_2 NP and free radical generation.

Control arterioles dilated in response to increasing concentrations of ACh (80±4%) and this dilation was impaired in the presence of L-NMMA (34±9%) or INDO (45±11%) as expected. Intravenous CeO_2 NP exposure significantly impaired dilation to ACh (11±10%) and this impairment was not altered after INDO or L-NMMA treatment. Pulmonary CeO_2 exposure also significantly impaired ACh dilation (30±4%). In addition, INDO treatment did not alter this impairment (44±10%); however, there was a partial restoration in function following L-NMMA treatment (51±11%). Smooth muscle activation was intact following both exposure routes. CeO_2 NP is capable of reacting with NO and there appears to be minimal free radical changes.

In conclusion, these results provide evidence that CeO_2 NP exposure impairs endothelial function, at least in part, via a NO-dependent mechanism and the mechanism of action may depend on exposure route.

Funding: NIH RO1-ES015022 (TRN), NSF IGERT (VCM), NIH F32-ES023435 (PAS)

SMALL AIRWAY EPITHELIAL CELLS EXPOSURE TO PRINTER EMITTED PARTICLES INDUCES CELLULAR EFFECTS ON HUMAN MICROVASCULAR ENDOTHELIAL CELLS IN AN ALVEOLAR-CAPILLARY CO-CULTURE MODEL

Jennifer D. Sisler[1], Sandra Pirela[2], Joseph Brain[2], Sherri Friend[1], Mariana Farcas[1], Diane Schwegler-Berry[1] Anna Shvedova[1], Treye Thomas[3], Vincent Castranova[1], Phil Demokritou[2], and Yong Qian[1]

[1]Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505, USA
[2]Center for Nanotechnology and Nanotoxicology, Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA
[3]Consumer Product Safety Commission, Office of Hazard Identification and Reduction, Bethesda, MD 20814

The printer is one of the most common pieces of equipment within an office space with multiple users. Recently, it was demonstrated that toner formulations for printing equipment incorporated engineered nanomaterials (ENM) which become airborne during printing and may cause adverse health effects when inhaled. To date, insufficient research has been performed to understand the toxicological properties of printer emission particles (PEPs). There have, however, been reports suggesting that toner nanoparticles can cause chronic inflammation and fibrosis within the lungs of rats. The present study investigates the toxicological effects of PEPs using an *in vitro* alveolar-capillary co-culture model with Human Small Airway Epithelial Cells (SAEC) and Human Microvascular Endothelial Cells (HMVEC). PEPs were collected from a commonly used office laser printer with the average number of particles emitted was 1.26 $x10^6/cm^3$. The size of the PEPs ranged from 39 to 122 nm and had a chemical composition of 42% carbon, 1.5% metal (Al, Ti, Ce, Zn, and Cu) and 56% other (phosphorus, sulfur, chlorine). Our data demonstrate that by direct exposure of SAEC to low concentrations of PEPs (1.0 µg/mL) caused morphological changes of actin remodeling and gap formations within the endothelial monolayer. The HMVEC increased production of reactive oxygen species (ROS) and exhibited increased angiogenesis. The analysis of cytokine and chemokine levels demonstrates that IL-6 may play a role within the toxicological effects seen between SAEC and HMVEC. These data indicate that PEPs are bioactive and effect cellular communication within the alveolar-capillary co-culture model possibly suggesting that they will interfere with cell-cell communication *in vivo* causing adverse health effects.

Disclaimer:
The findings and conclusions in this report are those of the authors and do not necessarily represent views of the National Institute for Occupational Safety and Health.

COMPARATIVE 'OMICS STUDIES OF SPRAGUE-DAWLEY RATS EXPOSED TO TIO_2 NANOPARTICLE AEROSOLS

Stephen J. Valentine,*[1] Gregory C. Donohoe,[1] Hossein Maleki,[1] Jinghai Yi,[2] Carroll McBride,[2] Timothy R. Nurkiewicz,[2] Chris Bolcato,[3] Yuan Xianglin,[3] Xinmin Yin,[4] Xiang Zhang,[4]

[1]C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV; [2]Center for Cardiovascular and Respiratory Sciences, West Virginia University School of Medicine, Morgantown, WV; [3]Protea Biosciences, Inc., Morgantown, WV

The increasing production of engineered nanomaterials (ENM) intensifies the need to understand the exposure effects upon human health. Although the cardiovascular system is impacted upon pulmonary exposure to ENM, molecular mechanisms linking the exposure site to the downstream effects are not fully understood. This study employs a systems biology approach to elucidate potential biopathways associating exposure and vascular events. Male Sprague Dawley rats were exposed to nano-titanium dioxide (TiO_2) aerosols for four to six hours per day to reach a target mass concentration of 6.0 mg/m^3. Plasma was generated from blood collected from organisms at two different post-exposure time points (0 and 24 hours). Controls were exposed to filtered air. Plasma samples were pooled from 23 separate organisms. Proteomics investigations were conducted by removing abundant proteins (albumin and IgG) and subjecting plasma tryptic digests to two-dimensional (2D) liquid chromatography (LC) and tandem mass spectrometry (MS/MS) analysis. Metabolite extracts were analyzed by 2D gas chromatography (GC×GC) coupled with MS as well as ion mobility spectrometry (IMS) coupled with MS/MS. Comparative analysis using PCA reveals at least 29 proteins that are up regulated or down regulated upon exposure and can be used to distinguish the two sample types. Metabolomics analysis yields 26 molecules with different abundances that can be used to differentiate the samples. A biopathway analysis of the 29 proteins and 26 metabolites produces a highly integrated regulation network. The network has been linked to inflammation and cell-to-cell signaling and interaction. IMS-MS results indicate differences in specific metabolite abundances (LysoPCs) that can be used to distinguish the two post-exposure time point samples. Considered together, these findings and markers represent strong evidence linking pulmonary ENM exposure and systemic microvascular dysfunction via inflammatory mechanisms.

Support: NIH-R01-ES015022 (TRN)

INTRATRACHEAL CO-EXPOSURE TO DIESEL EXHAUST PARTICULATE AND CRYSTALLINE SILICA IN RATS POTENTIATES THE INFLAMMATORY EFFECTS OF SILICA IN THE LUNGS

[1,2]BY Farris, [2]CE McLoughlin, [1,2]JS Fedan, [1,2]BT Chen, [2]D Schwegler-Berry, [1,2]JM Antonini, [1,2]JR Roberts

[1]WVU, Morgantown, WV; [2]NIOSH, Morgantown, WV

Worker exposure to high levels of respirable dust containing crystalline silica and diesel exhaust (DE) has become a concern during hydraulic fracturing operations. The goal of the current study was to investigate the effects of acute pulmonary co-exposures to silica and DE particulate (DEP) *in vivo*. Doses were derived from field measures using the high point values for respirable silica, applied to a human pulmonary deposition model, and normalized to the surface area of the rat lung. The silica dose of 230µg/rat represents 2.5 mg/m3 for 12 hr/d for 14 d. The DEP dose of 7.9 µg represents 0.1 mg/m3 for the same duration in a deposition model for total carbon (TC). A 50 µg dose of DEP was also used to represent ~0.6 mg/m3 TC for the same duration. Rats were exposed by a single intratracheal instillation of sterile PBS as vehicle or one of the following doses: 7.9 µg DEP, 50 µg DEP, 230 µg silica, or silica and DEP combined for each dose of DEP. At 1 d, 1 wk, and 1 mo post-exposure, bronchoalveolar lavage (BAL) was performed on the right lungs; cells and fluid were retained to assess pulmonary injury and inflammation. Lung-associated lymph nodes (LALN) were harvested to evaluate immune responses. Silica alone caused increased inflammation and lung injury at all times post-exposure, indicated by increased neutrophil influx, oxidant production, and lactate dehydrogenase (LDH) in BAL. DEP alone caused only slight inflammation 1 d post-exposure but no effects later. At 1 wk and 1 mo after exposure, 50 µg DEP significantly increased the inflammatory effects of silica. In addition, at 1 mo, both co-exposure doses significantly enhanced the phagocyte oxidant production over silica alone. Lymphocyte counts in LALN in the co-exposures were not elevated relative to silica exposure alone. In summary, DEP alone produced relatively no pulmonary toxicity; however, DEP in a co-exposure with silica has the capability to potentiate the adverse effects of silica in the lung.

Keywords: Diesel Exhaust Particles, Crystalline Silica, Lung Inflammation, Occupational Exposure, Oil Industry

Disclaimer: The findings and conclusions of this abstract have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

SPP1 MODULATION OF ICAM-1 REGULATES GENDER-SPECIFIC DIFFERENCES IN EARLY NEUTROPHIL RECRUITMENT TO THE LUNGS POST-SILICA EXPOSURE

Alexander Chukwuma Ufelle and Cheryl L. Fattman

Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, PA 15219

Background:

Inflammation is implicated in the pathogenesis of silicosis and animal models of silica-induced pulmonary fibrosis. We have demonstrated that silica-treated female mice recruit a greater number of inflammatory cells which are predominantly macrophages, produce lower levels of secreted phosphoprotein 1 (Spp1) and develop less pulmonary fibrosis compared to male mice at 14 days post-exposure. Neutrophils are the predominant inflammatory cell type recruited into the lungs after 3 days of silica exposure and intercellular adhesion molecule 1(ICAM-1) is induced after 24 h of silica exposure in mice. The role of gender and Spp1 in neutrophil recruitment at pre-fibrotic time points is unknown. We therefore hypothesized that female mice recruit greater numbers of neutrophils at pre-fibrotic time points and that Spp1 regulates ICAM-1 to mediate gender-specific differences in neutrophil recruitment to the lungs following exposure to crystalline silica.

Results:

Silica-exposed female mice recruited greater numbers of neutrophils and have more extensive lung injury compared to exposed males at 3 days post-treatment. In addition, silica-exposed female mice express greater levels of ICAM-1 mRNA compared to exposed males at pre-fibrotic time points. Furthermore, silica-exposed female mice express lower levels of Spp1 compared to exposed males at pre-fibrotic time points. To determine if Spp1 plays a role in neutrophil recruitment at 3 days post-exposure, we treated C57BL/6 (WT) and Spp1 (-/-) mice with silica. Spp1 deficiency abolishes the observed gender-specific differences in neutrophil recruitment and lung injury but does not influence overall neutrophil recruitment between WT and Spp1 (-/-) in either gender mice. Finally, Spp1 deficiency upregulates ICAM-1 mRNA expression at 3 days post-silica exposure.

Conclusion:

Spp1 levels are higher in male mice, and Spp1-mediated suppression of ICAM-1 causes fewer neutrophils to be recruited in males compared to females at 3 days post-silica exposure.

DEVELOPMENT AND USE OF INNOVATIVE AEROSOL SAMPLERS TO MEASURE AMBIENT EXPOSURES TO PARTICULATE MATTER

Gary Casucio

R.J. Lee Group
350 Hochberg Road, Monroeville, PA 15146

New and innovative approaches to sampling aerosols are constantly evolving. This presentation will focus on two aerosol sampling technologies that can be used to complement, and in some cases replace, existing methodologies. The first part of the presentation will focus on a passive sampling technology that was developed at the University of North Carolina (UNC). The UNC Passive sampler can be used to determine ambient concentrations (ug/m^3) of particulate matter (PM_{10} and $PM_{2.5}$) based on the measurement of the size (and composition) of the particles collected on the sampler. The second part of the presentation will focus on development of a nanoparticle-speciation sampler. This sampler uses thermophoretic force to collect particles (<300 nm) onto an electron microscope (EM) grid for subsequent analysis using EM techniques. The advantages and limitations associated with each of the samplers will be discussed.

PARTICLE CHARACTERIZATION AND TOXICOLOGICAL EVALUATION OF PULMONARY EXPOSURE TO GRAPHENES OF DIFFERENT SIZES

[1,2]JR Roberts, [1]A Kenyon, [1,2]RR Mercer, [1,2]T Sager, [1]M Wolfarth, [1]JF Scabilloni, [1,2]SS Leonard, [1]A. Stefaniak, [1]NR Fix, [1,2]BY Farris, [2]MS Seehra, [3]IS Chaudhuri, [3]A Kyrlidis, [1]SA Bilgesu, [1]D Schwegler-Berry, [1,2]DW Porter, [1]V Castranova, [1,2]A Erdely

[1]NIOSH, Morgantown, WV; [2]WVU, Morgantown, WV; [3]Cabot Corporation, Billerica, MA

Research on the uses and manufacturing of graphene nanomaterials has increased dramatically in the past decade. The process of manufacturing dry powder graphene may increase the risk of respiratory exposure to workers. The goal of this study was to evaluate the lung toxicity of three non-oxidized graphenes (Gr) of different sizes [20 µm lateral x 7-10 nm thick (Gr20), 5 µm lateral x 7-10 nm thick (Gr5), and <2 µm lateral x 1-2 nm thick (Gr1)]. Printex 90, a nanoparticle form of carbon black (CB), was used as particle control. Surface area (SA) and structural analysis was performed on the dry powder. Gr samples were then diluted in physiological dispersion medium (DM) and characterized for size, zeta potential, and surface reactivity in DM, and free radical generation in an *in vitro* system. Gr samples were found to be similarly composed of two forms of graphite structures (2H and 3R). Particle SA was similar for Gr20 and Gr5 (~107-120 m^2/g), while Gr1 had the greatest SA (~750 m^2/g) followed by CB (~330 m^2/g). Number of layers per sample of Gr was found to be inversely related to SA, with Gr20, Gr5, and Gr1, composed of 64-72, 75-84, and 28-30 layers, respectively. Aggregate size in DM ranged from ~ 5-300 µm, 0.5-60 µm, and 0.2-5 µm for Gr20, Gr5, and Gr1, respectively, with CB being similar to Gr1. Zeta potential did not differ significantly among particles. Electron spin resonance (ESR) indicated that all Gr samples and CB had low to no surface reactivity. *In vitro*, ESR showed all Gr20 and Gr5 samples induced greater free radical production by mouse monocytes as compared to Gr1- and CB-treated cells. Following characterization, male C57BL/6J mice received 4 or 40 µg of Gr1, Gr5, or Gr20, or 40 µg of carbon black (CB; particle control), or DM (vehicle control) by aspiration. Mice were sacrificed at 4 hr (0 d), 1, 7, 28, and 64 d post-exposure. In one group of animals, lung lavage was performed to assess indices of lung injury and inflammation. Aorta, heart, liver, and serum were collected for measures of systemic inflammation and oxidant stress. In a separate set of mice, the left lung was preserved for gene expression analysis and the right lungs were processed for histological and morphometric analysis. Indices of lung injury in lavage fluid were increased for the 40 µg doses of Gr20 and Gr5 on 0, 1, 7, and 28 d when compared to control. Injury in the CB group was comparable to Gr20 and Gr5 on d 1 and 7. Injury decreased significantly in all groups by 28 d. Lung inflammation and indices of oxidative stress in lung lavage were elevated in the 40 µg Gr20, Gr5, Gr1 and CB groups on d 1, and in the 40 µg of Gr20 and Gr5 groups on 0, 7, and 28 d, with resolution by 64 d in all groups. Increased inflammatory signaling, measured by relative mRNA expression, was increased for all groups initially. The effects returned to baseline by 7 d for Gr1 and all low Gr doses but remained increased (2-fold) in the 40 µg Gr5 and Gr20 at 64 d. Histopathological analysis showed only minimal to moderate epithelial hypertrophy and hyperplasia, as well as terminal bronchiolar inflammation, in areas only where particle deposited, and was slightly more severe for Gr 20 and 5. All particles were found to deposit in airways and alveoli, with a greater deposition of Gr20 and Gr1 in the airways versus Gr5 and CB. CB and Gr1 were cleared faster than the larger Gr particles. Systemically, inflammatory gene expression changes in the aorta at 0 d and liver acute phase genes at 1 d were only evident in the high doses excluding Gr1 with no marked differences in serum inflammatory protein levels. In summary, Gr deposition and clearance was dependent on the Gr size with Gr 5 being the most bio-persistent. The larger Gr particles appeared to produce more initial lung inflammation which persisted longer than that of the smaller Gr1 and CB particles.

POSTER ABSTRACTS

- ***Please note that posters must be placed on the corresponding board numbers listed on the following page.***
- ***Presenting authors must be present from 4:00 – 6:00 pm.***
- ***** *indicates posters are also presented in the Symposia listed in the preceding section (Presentation Abstracts, pages 7-16).*

FIRST AUTHOR	*AFFILIATION*	*BOARD #*
Badding, M.A.	NIOSH	1
Badding, M.A.	NIOSH	2
* Beezhold, K.	University of Pittsburgh	3
* Casucio, G.	R.J. Lee Group	4
Davidson, D.C.	NIOSH / West Virginia University / Harvard	5
Dunnick, K.	NIOSH / West Virginia University	6
* Engler-Chiurazzi, E.B.	West Virginia University	7
Eyoita, E.	West Virginia University / Ohio Co. Health Dept.	8
Fedan, S.	NIOSH / Shinshu University	9
Filbert, V.	Edinboro University	10
Horvath, K.	Indiana Area Senior High School / Indiana University of Pennsylvania	11
Knuckles, T.	West Virginia University	12
Mackay, C.	Duquesene University	13
Mandler, W.K.	West Virginia University	14
Manke, A.	West Virginia University / NIOSH	15
McLoughlin, C.E.	NIOSH	16
Mihalchik, A.L.	NIOSH	17
* Minarchick, V.C.	West Virginia University / NIOSH	18
Nichols, C.E.	West Virginia University / NIOSH	19
Palliyaguru, D.L.	University of Pittsburgh	20
* Pope-Varsalona, H.	University of Pittsburgh	21
Russ, K.A.	University of Michigan	22
Sager, T.M.	University of Montana / NIOSH	23
Shepherd, D.L.	West Virginia University	24
Shimko, M.J.	West Virginia University / NIOSH	25
Siegrist, K.	NIOSH / West Virginia University / Mayo Clinic / R.J. Lee Group	26
Snyder-Talkington, B.N.	NIOSH / West Virginia University	27
Stapleton, P.G.	West Virginia University	28
Stueckle, T.	NIOSH / West Virginia University	29
Thompson, J.A.	NIOSH / Cabot Corporation	30
Ufelle, A.C.	University of Pittsburgh	31
Wagner, L.M.	NIOSH / CDC	32
* Valentine, S.J.	West Virginia University	33
Zaccone, E.J.	West Virginia University / NIOSH	34
Zaccone, E.J.	West Virginia University / NIOSH	35

INDIUM-TIN OXIDE PRODUCTION PARTICLES INDUCE *IN VITRO* INFLAMMATORY RESPONSES, INCLUDING ACTIVATION OF THE NLRP3 INFLAMMASOME

Melissa A Badding[1], Natalie R Fix[1], Kristin J Cummings[2], and Stephen S Leonard[1]

[1]Health Effects Laboratory Division, NIOSH, Morgantown, WV, United States. [2]Division of Respiratory Disease Studies, NIOSH, Morgantown, WV, United States.

Lung disease among workers in the indium-tin oxide (ITO) industry is an emerging occupational health concern as the demand for consumer electronics continues to increase. Epidemiologic studies have shown indium compound-exposed workers may develop pulmonary alveolar proteinosis and fibrotic interstitial lung disease. Although both of these diseases are the result of an overactive inflammatory response, the molecular mechanisms behind indium compounds' toxicity and ability to initiate such diseases remain unknown. Thus, we aim to uncover how compounds encountered during ITO production affect cultured cells, and ultimately contribute to the pathogenesis of these diseases. Our preliminary studies showed that sintered ITO (SITO) exposures caused significant cell death in both RAW 264.7 monocyte macrophages and BEAS-2B bronchial epithelial cells. Additionally, nuclear factor kappa beta (NFκB) activation occurs within 3 hours of SITO treatment, followed by robust cytokine production (TNFα, IL-6, IL-1β, and IL-8) within 24 hours, confirming that pro-inflammatory responses are indeed occurring in both cell lines. These results point to potential NLRP3 inflammasome activation. This cytoplasmic multi-protein complex responds to an array of different stressors, but typically requires two separate signals for the production of pro-IL-1β (i.e., TLR signaling) and release of biologically active IL-1β (caspase-1 activation via ionic efflux). When various inflammasome components were blocked upstream or downstream of NLRP3, SITO-induced IL-1β production was significantly prevented. This suggests that SITO particles are able to activate the inflammasome and cytokine production without an additional TLR priming stimulus. Thus, it is possible that activation of the NLRP3 inflammasome plays a role in initiating and propagating indium lung disease. These findings have provided a better understanding of the molecular mechanisms behind an emerging occupational health issue and will aid in the discovery of potential biomarkers for exposed workers.

A COMPARISON OF *IN VITRO* CYTOTOXICITY AND OXIDATIVE STRESS FROM WELDING FUMES GENERATED WITH A NICKEL-, COPPER-BASED CONSUMABLE VERSUS MILD AND STAINLESS STEEL-BASED WELDING

Melissa A Badding, Natalie R Fix, Stephen S Leonard, James M Antonini

Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV, 26505, USA.

Welding processes that generate fumes containing toxic metals, such as hexavalent chromium (Cr(VI)), manganese (Mn), and nickel (Ni) have shown to cause lung injury, inflammation, and lung tumor promotion in animal models. While federal regulations have reduced permissible worker exposure limits to Cr(VI), this is not always practical considering that welders may work in confined spaces and exhaust ventilation may be ineffective. Thus, there has been a recent initiative to minimize the potentially hazardous components in welding materials by developing new consumables containing much less Cr(VI) and Mn. A new Ni and copper (Cu)-based material (Ni-Cu WF) is being suggested as a safer alternative to stainless steel consumables; however, its adverse cellular effects have not been evaluated. This study compared the cytotoxic effects of the newly-developed Ni-Cu WF with two well-characterized welding fumes, collected from gas metal arc welding using mild steel (GMA-MS) or stainless steel (GMA-SS) electrodes. RAW 264.7 mouse macrophages were exposed to the three welding fumes at two doses (50 µg/ml and 250 µg/ml) for up to 24 hours. Cell viability, reactive oxygen species (ROS) production, phagocytic function, and cytokine production were examined. The GMA-MS and GMA-SS fumes were found to be more reactive in terms of ROS production compared to Ni-Cu WF. However, Ni-Cu WF was more cytotoxic, inducing cell death and mitochondrial dysfunction at a lower dose. Additionally, pre-treatment with Ni-Cu WF particles impaired the ability of cells to phagocytize *E. coli*, suggesting macrophage dysfunction. Thus, the toxic cellular responses to welding fumes are largely due to the metal composition. The results also suggest that reducing Cr(VI) and Mn in the generated fume by increasing the concentration of other metals (e.g., Ni, Cu) may not necessarily improve welder safety.

IN VIVO/IN VITRO ANALYSIS OF THE EFFECTS OF THE PHYSICOCHEMICAL PROPERTIES OF NANO-SCALED CERIUM OXIDE ON FIBROGENESIS

Donna C. Davidson[*], Raymond Derk[*], Michael Chen[**], Todd A. Stueckle[*], Mark Barger[*], Jane Ma[*], Philip Demokritou[***], Vincent Castranova[*], and Liying Wang[*]

[*]National Institute for Occupational Safety and Health, HELD/PPRB, Morgantown, WV 26505; [**]West Virginia University, School of Pharmacy, Morgantown, WV 26505; [***]Harvard University, School of Public Health, Boston, MA 02115

Nano-scaled cerium oxide ($nCeO_2$) is used for a variety of applications encompassing numerous fields, such as the automobile industry, petroleum refining, optics, and mechanical and chemical polishing. In addition, these compounds have potential utility within the biomedical industry and as an additive in consumer products, such as sunscreens and cosmetics. *In vivo* studies have shown that inhaled $nCeO_2$ can deposit in deep lung tissues including the alveoli, the interstitium, and the pleural area. However, to date, most toxicological assessments of $nCeO_2$ have been limited in their size distribution as well as surface chemistry, and have primarily focused on alveolar epithelial cell responses to $nCeO_2$. Given the growing number and types of industrial and commercial uses of $nCeO_2$, it is imperative that hazard assessments be performed incorporating additional cell types and a broad range of sizes of $nCeO_2$, as well as various surface alterations (eg. amorphous silica coating). Thus, we hypothesize that the physicochemical properties of $nCeO_2$ may play an important role influencing the bio-activity of this engineered nanoparticle. Previous studies have demonstrated that $nCeO_2$ induces lung fibrosis in a rat model, with increased markers of inflammation or fibrogenesis, such as IL-12, TGF-β, and matrix metalloproteinases (MMPs) observed in the bronchoalveolar lavage fluid. Present data, obtained using plasma samples from the same $nCeO_2$ treated rats, indicates an increase in circulating levels of the fibrosis mediator TGF-β, which can be released from both macrophage and platelets upon stimulation, further supporting its involvement in $nCeO_2$-induced fibrogenesis, as well as demonstrating its potential as a bio-marker for $nCeO_2$ response. In addition, our data show that $nCeO_2$ is able to induce the production of collagen I, a hallmark of fibrogenesis, in lung fibroblast cells *in vitro*, further demonstrating the direct fibrogenic effect of $nCeO_2$. Little is currently known about how changes in physicochemical properties of $nCeO_2$ may affect the fibrogenicity of this compound. Thus, to address this knowledge gap, we are testing various sizes, as well as amorphous silica-coated $nCeO_2$ nanoparticles using several major lung cell types and subsequently analyzing cytokine release, oxidative stress induction, cytotoxicity, morphological changes, and functional tests in an effort to determine the fibrogenicity of a variety of $nCeO_2$ compounds. These studies are expected to provide novel information to identify health hazards as well as relevant *in vitro* tools to predict $nCeO_2$-induced inflammatory or fibrogenic activity.

Disclaimer: The findings and conclusions in this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

EVALUATING CERIUM OXIDE NANOPARTICLE CYTOTOXICITY

K. Dunnick[1], M. Badding[1], E. Sabolsky[2], and S. Leonard[1]

[1]Health Effects Laboratory Division, NIOSH, Morgantown, WV, United States.
[2]WVU Department of Mechanical Engineering, Morgantown, WV

Cerium oxide (CeO_2) nanoparticles are currently being used in a variety of applications, including catalysts, solar cells, gas sensors, and additives to diesel fuel. As a result of the diverse uses of CeO_2, manufacturing of products containing this nanomaterial is expected to increase. This increase will result in greater exposure risks, specifically inhalation risks, for individuals working in the manufacturing process. Therefore, to understand and prevent potentially toxic inhalation exposures, toxicity screening of CeO_2 is crucial. Current literature on CeO_2 toxicity is inconclusive and provides mixed results as to the degree of CeO_2 toxicity. Research has shown that CeO_2 promotes reactive oxygen species (ROS) production and inflammation, however other studies have shown that CeO_2 may actually protect against ROS and has antioxidant like properties. These conflicting results have been hypothesized to be the result of cerium's unique ability to exist in either a +3 or +4 valence state. Therefore, we hypothesize that by doping CeO_2 nanoparticles and subsequently forcing their overall valence state toward +3 would result in greater antioxidant like effects. RLE-6TN rat alveolar epithelial cells were used to assess CeO_2 toxicity. Preliminary studies were completed to assess cellular uptake over a time course of 9 hours using electron microscopy. ROS production was measured using a DCFH assay and dose dependent cytotoxicity of CeO_2 was measured using an MTT and Caspase 3/7 assay. Flow cytometry was implemented to assess apoptosis and necrosis following CeO_2 exposure at 24 and 48 hours. Electron microscopy showed accumulation of CeO_2 within cells by 1 hour at doses as low as 1 µg/ml. CeO_2 caused significant decreases in ROS levels at doses higher than 10 µg/ml compared to control treated cells. At doses higher than 50 µg/ml, significant decreases in cell viability were measured at 24 and 48 hours and by 48 hours, increased Caspase 3/7 was measured. Preliminary data shows that at high exposure levels, CeO_2 exerts toxic effects at 24 and 48 hours, which may be mediated through mitochondrial disturbances.

EFFECTS OF ULTRAFINE PARTICLE EXPOSURE FROM MARCELLUS SHALE GAS DRILLING OPERATIONS ON PEDIATRIC ASTHMA

Eyoita, Ekanem[1]; McCawley, Michael[1]; Chatterjee, Somu[2]; Mercer, William C.[2]; Harris, Wayland W.[2]; Knuckles, Travis L.[1]; Gamble, Howard P.[2]

[1]West Virginia University, Morgantown, WV, United States.

[2]Ohio County Health Department, Wheeling, WV, United States.

Marcellus Shale drilling operations utilize diesel engines, which result in a release of ultrafine particles (UFPs; particles less than 0.1 μm) during combustion processes. Recent epidemiological studies have found a positive association between UFPs and pulmonary health effects in humans. Furthermore, UFPs are of increasing concern because studies show they exacerbate asthma symptoms, especially in children. In this paper, we describe sampling conducted in Ohio County, West Virginia using a new, battery-operated, portable differential mobility analyzer to measure the concentration and size distribution of UFPs to predict health effects that may occur in areas where the wells are located. This allowed not only total particle number concentration to be determined but also the lung dose to be estimated based on established lung deposition models. We measured concentrations that averaged as low as 4,000 to as high as 46,000 particles per cm^3 in areas around drilling operations. These concentrations were generally found to be higher than the 8,000-particles/cm^3 concentrations associated with increased emergency room admissions for pediatric asthma. Moreover, these concentrations were consistent with ambient air particle counts of obtained near mountaintop mining operations, which have high levels of cardiovascular disease and cancer in the population. The concentrations of UFPs were similar between the two industrial sites. Collectively, these data suggest a substantial ultrafine particle exposure burden to the populace proximal to the mining and drilling operations, which may be driving some of the adverse respiratory and cardiovascular effects. Currently, the Environmental Protection Agency does not monitor ultrafine particle concentrations in these areas, nor are there proposed limits for exposure. Hence, a health-monitoring plan needs to be put in place with local hospitals to assure protection of the health of the population.

INHALATION OF MULTI-WALLED CARBON NANOTUBES (MWCNT) AFFECTS LUNG RESISTANCE AND COMPLIANCE AND EVOKES AIRWAY HYPERREACTIVITY TO METHACHOLINE IN RATS

Jeffrey S. Fedan[1], Janet A. Thompson[1], David G. Frazer[1], Dale W. Porter[1], Shuji Tsuruoka[2], Morinobu Endo[2], and Walter McKinney[1]

[1]Pathology and Physiology Research Branch, NIOSH, Morgantown, WV 26505
[2]Shinshu University, Nagano-shi, 380-8553 Japan

The incorporation of carbon nanotubes into new materials has raised concerns about their potential hazards to workers exposed during manufacturing. In animals, following pharyngeal aspiration, i.t. instillation and inhalation exposures to MWCNT, airway inflammation and lung fibrosis have been reported. However, the effects of inhalation of MWCNT on pulmonary function and reactivity to inhaled methacholine (MCh), a bronchoconstrictor, have not been studied. Therefore, we investigated whether inhaled MWCNT affect lung resistance (RL) and dynamic compliance (CDyn), and/or reactivity to inhaled MCh aerosol. Male rats were exposed for 6 h to filtered air or 5 mg/m3 of aerosolized MWCNT (MWNT-7; Hodogaya Chemical Co.; 20 - 50 walls; median length, 3.86 μm; mean width, 49 nm; mass median aerodynamic diameter, 1.5 μm; particle count aerodynamic diameter mode, ~0.4 μm). Basal RL and CDyn were measured 18 h and 7 d after the end of exposure, and responses to increasing concentrations of MCh were obtained. Eighteen h after exposure basal RL was increased and basal CDyn was decreased; 7 d after exposure basal RL and CDyn were not changed. Reactivity to MCh (RL) was increased and CDyn responses were decreased at 18 h but not 7 d after exposure. The results indicate that lung function and airway reactivity changes occur acutely, but that these changes subside over time following a single MWCNT inhalation exposure.

INACTIVATION OF PATHOGENS ON THE SURFACES OF STERILE SWABS, FRUITS AND VEGETABLES USING SANITIZING SUBSTANCES PRODUCED BY RADIANT CATALYTIC IONIZATION

Victoria Filbert, Alicia Hipwell, Joseph Manozzi, Khadijat Okasime, Tikira Saunders, Jordanna Wallace, (Drs. William MacKay, David Fulford, Craig Steele)

Department of Biology and Health Services, Edinboro University, Edinboro, PA 16444

Foodborne illness outbreaks linked to fresh products are becoming more frequent and widespread. The United States Department of Agriculture has estimated the costs associated with foodborne illnesses to be between $2.3 billion and $4.6 billion a year. The areas impacted include fruits and vegetables, meats, seafood, poultry, baking, canning, and dairy industries. Reducing pathogens and additional microbial contamination on food contact surfaces will improve the quality and shelf life of many food products. New sanitizing technologies have emerged in recent years and are being widely used in a multitude of places to better decontaminate contact surfaces. Historically, both ozone-and peroxide-based technologies have been used as disinfectants in numerous applications. Radiant Catalytic Ionization (RCI) technology, particularly ozone, is thought to be safe to humans and the use of ozone is now considered to be an organic form of treatment to disinfect food contact surfaces. RCI technology has been widely accepted within the food processing industry during recent years. Ozone and hydrogen peroxide, generated by RCI, has countless applications for reducing the number of bacteria. This study has focused on the potential use of oxidative gases, including ozone and peroxide, generated by an RCI photocell for the inactivation of *Escherichia coli, Listeria inocula, Pseudomonas aeruginosa, Salmonella typhimurium*, and *Staphylococcus aureus*, introduced on the tips of sterile cotton swabs, and a number of fruits and vegetables which include apples, spinach, strawberries and cantaloupe. Our results indicate a 90% killing of bacteria with a 90 minute exposure to RCI, demonstrating that the low level of oxidative gases produced by RCI has the potential to be an effective surface disinfectant tool for use in food processing.

NITRITE INDUCED METHEMOGLOBIN FORMATION IN AQUATIC LARVAE OF THE MIDGE (*CHIRONOMUS DILUTUS*)

Kylie Horvath[1] and Thomas Simmons[2]

[1]Indiana Area Senior High School, Indiana Area School District, Indiana, PA 15701
[2]Department of Biology, Indiana University of Pennsylvania, Indiana, Pennsylvania 15705

Aquatic midge larvae of the genus *Chironomus* are unusual in having extracellular low molecular weight monomeric and dimeric hemoglobin in their hemolymph. This adaptation presumably allows them to survive the hypoxic conditions of muddy nutrient-enriched sediments. We tested the hypotheses that nitrite reversibly oxidizes hemoglobin to methemoglobin in midges, and that they have enzymatic and nonenzymatic mechanisms to maintain hemoglobin in the reduced state as in many vertebrates including humans. The lethal effects of nitrite were investigated in 4th instar larvae of *Chironomus dilutus* using ASTM and USEPA static renewal toxicity test methods. The LC_{50} for 48 h exposure to nitrite was 228 mg/L, whereas 24 h exposure caused less than 75% mortality up to 500 mg/L. Volume-dependent and heat labile NADH ferricyanide reductase (methemoglobin reductase) activity was detected in 0.6% (w/v) NaCl hemolymph extract. The sublethal effects of nitrite on total hemoglobin concentration, percent methemoglobin, NADH ferricyanide reductase activity, and glutathione level were assessed by exposing midges to 1, 10 and 100 mg/L nitrite for 24 h and then transferring to control water for a 48 h recovery period. After exposure to 100 mg/L nitrite, a 21% decrease in total hemoglobin concentration, a 13-fold increase in percent methemoglobin, and an 85% reduction in total glutathione level were observed, and they returned to baseline after 24 h of recovery. NADH ferricyanide reductase activity was unaffected and glutathione disulfide was undetectable under all conditions. In conclusion, nitrite oxidizes hemoglobin to methemoglobin and the enzyme methemoglobin reductase reverses its oxidation in *C. dilutus* as in vertebrates, indicating that this mechanism is evolutionarily conserved. Glutathione may also play a role in this reduction, but further study is needed. The ecological relevance of nitrite-induced hemoglobin oxidation is limited since the concentrations needed to elicit a response are higher than found in the environment. Lastly, the utility of *C. dilutus* as an invertebrate model to study methemoglobinemia should be explored.

INHALED NANO-TiO_2 ALTERS CARDIOVASCULAR AUTONOMIC FUNCTION

T Knuckles, J Yi, and T.R. Nurkiewicz

Center for Cardiovascular and Respiratory Sciences, West Virginia University, Morgantown WV 26506

Nanotechnology is a rapidly growing field with the potential to influence all aspects of modern life. However, the cardiovascular toxicity of nanomaterials is not well understood. We have previously demonstrated a disruption in normal microvascular function following nanomaterial exposure via alterations in autonomic control. Presently, using a telemetry and a baroreflex function model, we show changes in autonomic control following inhalation exposure to nanosized titanium dioxide (nano-TiO_2) for 4 hours for 2 days at 6 mg/m^3. Telemeterized rats were acclimatized and exposed to sham (day 0), then nano-TiO_2 or sham on 2 consecutive days (days 1, and 2). Renal sympathetic nerve activity (rSNA), mean arterial pressure (MAP) and heart rate (HR) were continuously monitored. 24 h after the last exposure, baroreflex sensitivity was measured with phenylephrine (PE; 1-8 µg/kg) or sodium nitroprusside (SNP; 5-20 µg/kg). MAP increased during nano-TiO_2 relative to day 0 and sham (max ΔMAP sham -8.1±4.3 mm Hg, nano-TiO_2 3.0±2.1 mm Hg). The nano-TiO_2-induced MAP increases were largely driven by diastolic blood pressure (DBP) increases (day 1: sham DBP 98±3.5 mm Hg, nano-TiO_2 110±2.3 mm Hg), suggesting an enhanced peripheral resistance. Overnight, rSNA and HR were significantly elevated following exposure, suggesting an augmentation in the normal sympathetic nerve diurnal pattern (max; ΔHR sham 3±5 BPM, nano-TiO_2 14±1 BPM: ΔSNA sham -2.2±0.9 µV, nano-TiO_2 0.84±0.6). PE infusion enhanced and prolonged the depression in HR following nano-TiO_2 exposure compared to sham (max ΔHR - sham 13±1 BPM, nano-TiO_2 -15±1), suggesting increased parasympathetic signaling, which could be mitigated with hexamethonium or atropine. Nano-TiO_2 exposure significantly enhanced MAP depression by SNP (max ΔMAP -34±1.5 mm Hg sham, -41±1.3 mm Hg nano-TiO_2), though the mechanism unclear. Considered together, engineered nanomaterial exposure may alter cardiovascular function through autonomic signaling that is not obvious under resting conditions. (Funding NIH ES015022, TRN)

ANALYSIS OF PLANT EXTRACTS THROUGH ANALYTICAL METHOD: GC-MS AND LC-MS/MS

Caroline Mackay and Stephanie J. Wetzel

Duquesne University, Department of Chemistry and Biochemistry

Plants are a major component of the environment and range from over 375,000 species. The compounds that specifically comprise each species and that distinguish one species from another are unknown and are not adequately documented. A library of plant constituents has not been made because the first step on analysis has not been perfected: the method in which one extracts the compounds from the plant. In this project, plants were analyzed on various analytical instruments: GC-MS and LC-MS/MS. A pilot study was performed on the LC-MS/MS using only one extraction method to determine that separation was possible. The plant samples used for the pilot study were taken from Hemigraphis Alternata ("Purple Waffle") and Sanderiana. The extraction solutions that were used are methanol and ethanol. Once the pilot study concluded, the extraction solution and parameters were varied on the GC-MS to produce the greatest separation. Extraction solutions that were used are as follows: ethanol, methanol, and chloroform. Samples of plants were taken from Aloe Vera, Purple Waffle, and Sanderiana. A blank was run with the samples to serve as the control. The spectra were analyzed through the GC-MS library. A repeatable extraction method and optimized GC-MS method will be performed.

THROMBOSPONDIN-1 ALTERS SKELETAL MUSCLE MICROVESSEL REACTIVITY AND IS ELEVATED FOLLOWING NANOMATERIAL EXPOSURE

W. Kyle Mandler[1], Timothy R. Nurkiewicz[2], I. Mark Olfert[1]

West Virginia University School of Medicine Department of Exercise Physiology[1], Department of Physiology and Pharmacology2, Center for Cardiovascular and Respiratory Sciences, Morgantown, WV 26505

The extracellular matrix protein thrombospondin-1 (TSP-1), is an *antagonist* of nitric oxide-mediated vascular relaxation by preventing activation of guanylyl cyclase and other downstream targets. Previous work by our group has shown decreased NO bioavailability and endothelial dependent vasodilation following engineered nanomaterial exposure. Our objective is to determine the effects of loss of TSP-1 on microvascular reactivity as a potential model to explore mechanisms behind nanomaterial endothelial toxicity.

Arteriolar responses to acetylcholine, phenylephrine, and adenosine, were assessed in skeletal muscle microvasculature of global TSP-1 null (KO) and wildtype (WT) littermate mice using whole-bath intravital microscopy and targeted vessel iontophoresis skeletal muscle. Under general anesthesia, the right gluteus maximus muscle was surgically externalized and placed in a superfusate bath of physiologic saline solution with care to preserve feed vasculature and innervation. For the whole-bath preparation, following a 30 minute equilibration period, bath concentrations of 10^{-6}, 10^{-4} and 10^{-2}M of acetylcholine or phenylephrine were added, with 10 minute washout periods between each dose, in a random order. Following acetylcholine and phenylephrine, adenosine was added at a concentration of 10^{-3}M to determine maximal passive dilation. For targeted-vessel iontophoresis, a micropipette with an opening of 1-2nm was filled with a 10^{-2}M acetylcholine. The tip of the pipette was guided under microscope to a point adjacent to the adventitia of the target microvessel. During this time a holding current of -200nA was applied to bath to prevent acetylcholine leakage from the pipette. Once in place and following a 20 minute equilibration period, Ejection currents of 5nA, 20nA, and 80nA were applied to the bath for 10 minutes each in a randomized order, with a 10 minute washout period between each step. Vessel diameters were measured three times in the final minute of each ejection period or equilibration period.

KO animals demonstrated 18.6%, 16.5% greater vasodilatory response to acetylcholine concentrations of 10^{-4} and 10^{-2}M. Using iontophoresis, KO animals exhibited 21% and 62% greater vasodilation at acetylcholine ejection currents of 20nA and 80nA, respectively. WT animals had a 23.8% greater vasoconstriction in response to the highest dose of phenylephrine, 10^{-3}M. KO animals demonstrated a 23.5% higher maximal dilatory capacity in response to 10^{-2}M adenosine than WT. These data establish the inhibitory role of TSP-1 for in vivo microvessel vasodilation and provide a model for elucidating the mechanism behind nanomaterial-induced endothelial dysfunction in the microvasculature.

Support: NIH R01-ES015022 (TRN) and NSF-1003907 (TRN, WKM).

ROLE OF STEM-LIKE CELLS IN NANOMATERIAL-INDUCED PULMONARY FIBROSIS

Amruta Manke[1], Sudjit Luanpitpong[1], Yong Yang[2], Liying Wang[3], Yon Rojanasakul[1]

[1]Department of Pharmaceutical Sciences, [2]Department of Chemical Engineering, West Virginia University, WV; [3]Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, WV.

Nanomaterials including carbon nanotubes (CNTs) have revolutionized the industrial field with their rapidly growing biomedical and commercial applications. However, the risk of adverse health effects of these nanomaterials is largely unknown. Recent studies have shown that CNTs can induce pulmonary fibrosis, a fatal and incurable lung disease, but the underlying mechanism is unclear. Since fibrosis is associated with aberrant tissue repair and extracellular matrix (ECM) accumulation, identifying the cells that are responsible for the repair and ECM production is fundamental to the understanding of fibrosis mechanism. We hypothesize that CNTs induce fibroblast stem-like cells (FSCs) and that such induction is essential to the development of fibrosis. Using fluorescence-activated cell sorting technique, we have demonstrated for the first time that CNTs can induce FSCs from normal human lung fibroblasts, as evidenced by their side population (SP) property and expression of stem cell markers, ABCG2 and ALDH1A1. These cells, isolated from SP-positive FSCs, showed a high expression level of type I collagen and α-smooth muscle actin, which are key biomarkers of fibrosis, as compared to non-SP cells. The induction of FSCs by CNTs was further shown to be redox-sensitive since inhibition of ROS generation by antioxidants effectively inhibited the FSC induction by CNTs. Together, our results indicate the induction of FSCs by CNTs and its role in fibrogenesis. This novel finding provides a new insight into the mechanisms of fibrosis and may aid in the development of early detection biomarkers and treatment strategies for the disease. [Supported by NIH grant R01-HL095579 and NSF grant EPS-1003907]

TOXICITY AND ALLERGY RESPONSES IN THE LUNG FOLLOWING PULMONARY EXPOSURE TO NANOPARTICLE SILVER IN MICE

C. E. McLoughlin[1], S. Anderson[1], K. A. Roach[1], K. Anderson[1], D. Schwegler-Berry[1], B.T. Chen[1], J. R. Roberts[1]

[1]NIOSH, Morgantown, WV

Silver nanoparticles (AgNP), due to antimicrobial properties, are widely used in medical applications and consumer products. Expansive use of AgNP in manufacturing raises the concern of effects following respiratory exposure in workers. Previous work in the laboratory has shown dose-dependent lung toxicity with inflammation and alterations in lung immune parameters in rodents. The goal of the current study is to characterize effects of AgNP for potential exacerbation/attenuation of respiratory allergy in an ovalbumin (OVA)-induced allergy model in BALB/c mice. For range-finding (RF) studies, mice were exposed to physiological dispersion medium (DM), 6.1μg (LO), 18.2μg (ME), or 73μg (HI) AgNP. AgNP were 20 nm diameter with 0.3% wt polyvinylpovidone coating (NanoAmor, Inc.), were suspended and sonicated before exposures by pharyngeal aspiration (PA). For RF studies assays were conducted on days 1, 10, and 29 post exposure—time points chosen to correspond with the allergy paradigm time course. Airway hyperreactivity was measured as Penh, bronchalveolar lavage (BAL) was performed on the whole lung, cells and fluid were retained for analysis of lung-associated injury and inflammation and phenotyping by flow cytometry, and lymph nodes (LN) were harvested for enumeration and phenotyping. Changes in Penh did not occur with AgNP alone at any time point. Results indicated a dose-dependent injury and inflammation by day 10 which began to resolve by day 29. For the allergy model, DM and OVA served as controls and ME and HI doses were chosen for study. Animals received i.p. injections of OVA + aluminum hydroxide gel (alum) during the sensitization phase on days 1 and 10. To elicit an OVA-specific response, 2 PA challenges of OVA were given on days 19 and 29. AgNP show a trend towards enhancing airway reactivity over OVA control as evidenced by increased Penh; however, cellular responses in the lung did not appear to be significantly enhanced beyond that of the OVA control. Lung-associated lymph node total cell numbers were increased with the high dose of AgNP. IgE levels in both serum and BAL were not increased with AgNP over OVA control. Results indicate AgNP may have a moderate effect on airway hyperreactivity response in the lung, but do not significantly alter the course of allergy development. Future work includes administering AgNPs following the sensitization phase to determine effects on the elicitation phase of allergic response.

NITROGEN-DOPED MULTI-WALLED CARBON NANOTUBE-INDUCED EFFECTS IN HUMAN SMALL AIRWAY EPITHELIAL CELLS

Amy L. Mihalchik, Colleen McLoughlin, Diane Schwegler-Berry, Mariana Farcas, Anna Shvedova, Dale Porter, Shuji Tsuruoka, Morinobu Endo, Vincent Castranova, and Yong Qian

Health Effects Laboratory Division, NIOSH, Morgantown, WV, United States.

Multi-walled carbon nanotubes (MWCNT) have been valued for their unique physicochemical properties, including their ability to be functionalized. The growing use of MWCNT and their derivatives in academic and industrial settings has raised the need to efficiently determine their potential toxicity. Nitrogen-doped MWCNT (ND-MWCNT) are modified MWCNT that have enhanced electrical properties and are used in a variety of aerospace and fuel cell applications. Although similar to MWCNT, the biocompatibility and mechanism of action of ND-MWCNT have yet to be elucidated. Recent *in vivo* data showed that ND-MWCNT induced inflammation and fibrosis in mouse lungs. Here, we assessed the uptake of ND-MWCNT into human small airway epithelial cells (SAEC) to determine their toxicological effects. ND-MWCNT were engulfed by SAEC through 24h. We showed that ND-MWCNT induced reactive oxygen species (ROS) production and phospho-tyrosine activation in SAEC over a 24h *in vitro* time-course. Furthermore, significant alterations to the cell cycle were observed in cells treated with ND-MWCNT, as shown by a decreased percentage of cells in the S phase. Confocal images showed altered localization of acetylated tubulin in ND-MWCNT treated cells. Additionally, a multiplex assay for inflammatory cytokines showed activation of various inflammatory cytokines including IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-17, MCP-1, and IFN-γ. Our results indicate that exposure to ND-MWCNT induces toxicological effects in SAEC. The results from this study may contribute to dissecting the molecular mechanisms involved in ND-MWCNT toxicity.

PULMONARY EXPOSURE TO CARBON-BASED NANOMATERIALS INDUCES SPATIALLY-DISTINCT CARDIAC MITOCHONDRIAL DYSFUNCTION

Cody E. Nichols[1], Aaron Erdely[2], Danielle L. Shepherd[1], Dharendra Thapa[1], Rebecca Salmen[2] Colleen McLoughlin[2], Tina Sager[2], Jenny R. Roberts[2], John M. Hollander[1]

[1]Division of Exercise Physiology, West Virginia University, [2]NIOSH, Morgantown, WV

While carbon-based nanomaterial use continues to grow, the health effects are not fully realized. Nanomaterial inhalation affects lung mitochondria, but cardiac mitochondrial effects are not well defined. Within the heart, analyses are complicated by the presence of two mitochondrial subpopulations: the subsarcolemmal (SSM), beneath the sarcolemma, and interfibrillar (IFM), between the myofibrils. The current study investigated the impact of pulmonary exposure to various carbon-based nanomaterials on the function of cardiac mitochondrial subpopulations. Male C57BL/6 mice were exposed using pharyngeal aspiration to either 40 µg of <2 µm x <2 µm x 1-2 nm graphene (GP-1), 5 µm x 5 µm x 7 nm (GP-5), multi-walled carbon nanotubes (MWCNT), or carbon black; 10 µg of MWCNT; or sham, dispersion media. Four or 24 h after exposure, cardiac mitochondrial subpopulations were isolated and run through polarographic assessments for mitochondrial oxygen consumption. Mitochondrial hydrogen peroxide (H_2O_2) production was measured using the fluorescent dye Amplex red. The IFM of mice exposed to GP-1 had decreased active respiration 24 h after exposure compared to shams ($P<0.05$). Exposure to GP-1, GP-5, and carbon black increased IFM H_2O_2 production compared to shams 4 h post-exposure ($P<0.05$). Both doses of MWCNT decreased active respiration rates in the IFM at 4 h compared to controls ($P<0.05$), but no difference at 24 h. Increased production of IFM H_2O_2 were seen 24 h after exposure to 40 µg MWCNT as compared to shams ($P<0.05$). No effects were observed in any SSM population. In conclusion, cardiac mitochondrial dysfunction was observed solely in the IFM following pulmonary exposure to carbon-based nanomaterials. Decreased function and increased H_2O_2 production in the mitochondria between the myofibrils can promote cardiovascular dysfunction.

SUPPORT: AHA 13PRE16850066; NIH DP2DK083095; NSF DGE1144676; NIH T32HL090610; NIOSH NTRC

WITHAFERIN A IS AN EXTREMELY POTENT INDUCER OF CYTOPROTECTION MEDIATED BY THE NRF2-KEAP1 SIGNALING PATHWAY

Dushani L. Palliyaguru[1], Dionysios Chartoumpekis[2], Shivendra Singh[2], Thomas W. Kensler[2]

[1]Department of Environmental and Occupational Health, [2]Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA

The **Nrf2-Keap1 signaling pathway** is a master transcriptional regulator of the antioxidant response, which guards cells and organisms against carcinogenesis, mutagenesis and other forms of toxicity. This phenomena is achieved by the induction of enzymes involved in the metabolism of toxins, particularly enzymes such as glutathione S-transferases (GST), glutamate-cysteine ligase (GCL), NAD(P)H:quinone reductase (NQO1) and heme oxygenase (HO-1) that facilitate the detoxication and elimination of carcinogens. Several classes of electrophilic chemicals, including phytochemicals present in certain spices, fruits and vegetables have been identified as activators of the Nrf2-Keap1 pathway. *Withania Somnifera* is a highly revered botanical agent that has been used in traditional Indian medicine for thousands of years. Recent studies have shown that withanolides such as **withaferin A (WA)** present in the leaves and root of the *Withania Somnifera* plant display disease therapeutic activity against cancer, diabetes, Alzheimer's disease and Parkinson's disease in several experimental models. **Our central hypothesis was that WA is a potent inducer of the Nrf2-Keap1 signaling pathway that can protect cells and organisms from toxic insult and prevent disease occurrence**. Our results using quantitative real-time PCR (RT-PCR) show that WA is much more potent and efficacious than other naturally-occurring, dietary agents in inducing transcriptional expression of cytoprotective enzymes in human mammary epithelial MCF10A cells. In experiments conducted to show that WA targets the Nrf2-Keap1 pathway, Nrf2 target gene induction was >5-fold higher in wild type mouse embryonic fibroblasts (WT MEF) treated with WA compared to their Nrf2-/- counterparts. Western blotting and RT-PCR data show that wild type C57BL/6J mice orally gavaged with 7 mg/kg WA induces significant cytoprotective gene expression in liver, small intestinal epithelium, lung, whole colon and brain compared to controls. Our studies also demonstrate that WA administered mice are profoundly protected from acetaminophen-induced hepatotoxicity as observed through attenuated serum alanine aminotransferase (ALT) levels and lowered zonal liver damage in Immunohistochemistry analyses. We have thus characterized WA as an extremely potent inducer of cytoprotection mediated by the Nrf2-Keap1 signaling pathway that protects cells and organisms against toxic injury and disease onset.

EFFECT OF C_{60} FULLERENES IN RAW 264.7 IMMORTALIZED MACROPHAGES

Kristen A. Russ, Ariel Dews, Myah Ray, Brandon Schneider, Tessa Adzemovic, Cameron Sawyer, Martin Philbert

Toxicology Program, School of Public Health, University of Michigan

C_{60} fullerenes are spheres of 60 carbon atoms that have a diameter of approximately 1 nanometer. Fullerenes, also known as “bucky balls,” can be produced in natural events such as forest fires or volcanic eruptions, but are also produced in large quantities for potential use in consumer products. In order to determine the effect fullerene exposure may have on immune cells of exposed populations, RAW 264.7 immortalized macrophages were exposed to C_{60} fullerenes. Transmission electron microscopy performed on macrophages exposed to terbium endohedral fullerenes showed the fullerenes were endocytosed by the macrophages, present in the cytosol unbound by vesicles, and seen entering the nucleus. Endocytosis inhibition studies, utilized to determine if endocytosis was a main mechanism of fullerene uptake by RAW 264.7 macrophages, showed no significant changes in fullerene presence in the cell when compared to control cells. Freeze fracture transmission electron microscopy, utilized to determine if fullerenes cross the lipid bilayer to gain entry into the cell, showed fullerenes near the membrane space on both the extracellular and intracellular sides of the membrane. Due to the fullerene’s ability to enter the cells, assays were utilized to determine if fullerenes were toxic to the cells at 24 hours. DNA fragmentation assays and annexin V/propidium iodide staining revealed no cell death at 24 hours. 5-Bromo-2’-deoxyuridine assays indicated decreases in cellular proliferation at 1 and 4 µg/mL C_{60}. Altogether, the data suggests that fullerenes are able to enter the cell via endocytosis, are potentially able to move through the lipid membrane to exist in the cytosol unbound by vesicles, and are able to enter the nucleus. Fullerenes do not cause apoptotic or necrotic death, but decrease proliferation at 1 and 4 µg/mL, suggesting that 24 hour fullerene exposure results in altered cellular function.

EFFECTS OF NANOPARTICLE PRE-EXPOSURE DISPERSION STATUS ON BIOACTIVITY IN THE MOUSE LUNG

Tina M. Sager[1, 2], Michael Wolfarth[2], Vincent Castranova[2], and Andrij Holian[1]

University of Montana, Center for Environmental Health Sciences, Missoula, Montana[1]

National Institute for Occupational Safety and Health, Morgantown, West Virginia[2]

Nanotechnology is emerging as one of the world's most promising new technologies. Due to the overwhelming growth of the nanotechnology field, health risk assessment for both workers and consumers is warranted. From a toxicology perspective, nanoparticles possess two features that promote their toxicity. The first feature involves physical-chemical characteristics of the nanoparticle, which include the surface area of the nanoparticle. The second feature is the ability of the nanoparticle to traverse cell membranes. These two important nanoparticle characteristics are greatly influenced by placing nanoparticles in liquid medium prior to animal exposure. Nanoparticles tend to agglomerate and clump in suspension, making it difficult to accurately deliver them for in vivo or in vitro experiments. Thus, we hypothesize that the nanoparticle dispersion status will correlate with the in vivo bioactivity/toxicity of the particle. The proposed questions of this study are of great importance to the nanotechnology/toxicology community, namely the highly debated question of whether dispersion status of the nanoparticle suspension (pre-exposure) is of importance, will be answered. Therefore, the questions being answered by this study will provide much needed insight into the area of nanoparticle bioactivity and toxicity. To test our hypothesis, nano-sized nickel oxide was suspended in four different dispersion media (phosphate buffered saline (PBS), dispersion medium (DM), Survanta, and Pluronics). At each respective dose, well-dispersed and poorly dispersed (suspensions were sonicated on ice utilizing a Branson Sonifer 450, 25W continuous output, 20 min or 5 min, respectively) suspensions were created. Mice (male, C57BL/6J, 7 weeks old) were given 0-80 µg/mouse of nano-sized nickel oxide in the different states of dispersion via pharyngeal aspiration. At one and seven days post-exposure, mice underwent whole lung lavage (WLL) to assess pulmonary inflammation and injury as a function of dispersion status, dose, and time. The results show that pre-exposure dispersion status correlates with pulmonary inflammation and injury. In fact, the nanoparticle/dispersion media combination that produced the smallest hydrodynamic particle size (nano-nickel oxide suspended in DM and sonicated for 20 minutes had an average hydrodynamic size of 7.5 nm) produced a greater increase in WLL polymorphonuclear leukocytes, lactate dehydrogenase activity, as well as albumin levels in WLL fluid than the other nano-nickel/suspension media combinations. These results indicate that a greater degree of pre-exposure dispersion increases pulmonary inflammation and cytotoxicity, as well as decreases in the integrity of the blood-gas barrier in the lung, respectively.

This work was supported by NIH grant F32 ES021341.

Keywords: Nanotoxicology, Pulmonary Toxicity, Inflammation, Cytotoxicity, In Vivo Studies

A DIFFERENT VIEWPOINT ON MITOCHONDRIAL DYSFUNCTION IN THE TYPE II DIABETIC PATIENT

Danielle L. Shepherd[1], Tara L. Croston[1], Anthony A. Holden[2], Kevin J. Tveter[2], Dharendra Thapa[1], Cody E. Nichols[1], Dustin M. Long[3], I. Mark Olfert[1] and John M. Hollander[1]

[1]Division of Exercise Physiology, Center for Cardiovascular and Respiratory Sciences, West Virginia University School of Medicine, Morgantown, WV. [2]Department of Surgery, West Virginia University School of Medicine, Morgantown, WV. [3]School of Public Health Department of Biostatistics, West Virginia University, Morgantown, WV.

Cardiac complications, including diabetic cardiomyopathy, are the leading cause of morbidity and mortality among diabetic patients. The mitochondria within the cardiomyocytes have been implicated in the etiology of both type 1 and type 2 diabetes mellitus, leading to cardiac complications, such as diabetic cardiomyopathy. Two distinct mitochondrial subpopulations exist within the cardiomyocyte; the subsarcolemmal mitochondria (SSM), located directly beneath the sarcolemmal membrane, and the interfibrillar mitochondria (IFM), situated between the myofibrils. We have previously observed enhanced dysfunction to cardiac SSM in db/db mice resulting from type 2 diabetes mellitus. The goal of this study was to determine whether hyperglycemia or hemoglobin A1c (HbA1c) level in type 2 diabetic patients correlates with the extent of SSM dysfunction observed in these patients. Cardiac mitochondria were isolated from atrial appendage of both non-diabetic and diabetic patients. Using linear spline models to assess correlative risk between hyperglycemia or HbA1c level and type 2 diabetes mellitus, we determined that type 2 diabetic patients display dysfunctional state 3 respiration rate and ETC complex I activity in the SSM irrespective of the absolute blood glucose or HbA1c level. In addition, we found that type 2 diabetic patients whom also possess comorbidities, such as coronary artery disease (CAD) and hypertension, display SSM dysfunction that is not due to either CAD or hypertension independently. Further, we assessed the body mass index (BMI) of the patient to determine if it served as an effective predictor of mitochondrial dysfunction. Using a scatter plot analysis of non-diabetic and type 2 diabetic patients' BMIs versus mitochondrial functional analyses, we found that BMI is not effective predictor of cardiac mitochondrial dysfunction for the type 2 diabetic patient. Finally, we found that the degree of mitochondrial dysfunction in type 2 diabetic patients remains consistent despite the extent of elevated blood glucose or HbA1c level. These findings suggest that independent of comorbidities cardiac SSM dysfunction is present in the type 2 diabetic patient heart and the degree of dysfunction is consistent independent of elevated blood glucose or HbA1c level.

NERVE GROWTH FACTOR REDUCES AMILORIDE-SENSITIVE NA^+ TRANSPORT IN HUMAN AIRWAY EPITHELIAL CELLS THROUGH A TRKA-ERK1/2 MEDIATED PATHWAY

Michael J Shimko[1], Eric J Zaccone[1], Janet A Thompson[2], Jeffrey S. Fedan[2].

[1]Department of Pharmaceutical and Pharmacological Sciences, West Virginia University, Morgantown, WV and [2]Pathology and Physiology Research Branch, NIOSH, Morgantown, WV

Nerve growth factor (NGF) is over-expressed in airways of patients with inflammatory lung diseases. Ion transport by the epithelial cells of the lung is responsible for controlling the periciliary liquid layer, and changes in ion transport can accompany disease. To investigate NGF's effects on airway epithelium, human primary cultured epithelial cells were placed in an Ussing chamber to measure short-circuit current (I_{sc}). NGF (1 ng/ml), applied apically, reduced I_{sc} (4.5%) and reduced amiloride (apical, 3.5×10^{-5} M) -sensitive Na^+ transport (control, 52%; NGF-treated, 38%). To investigate whether the NGF receptor, trkA, is responsible for mediating the responses to NGF, cells were incubated with either the tyrosine kinase inhibitor K-252a (10 nM, apical) or DMSO (0.004 %) in the absence or presence of NGF. K-252a did not affect Na^+ transport, but attenuated the NGF-induced reduction in Na^+ transport. To investigate whether trkA activates the Erk1/2 signaling cascade, which results in the down-regulation of ENaC, cells were incubated with PD-98059 (30 µM, apical and basolateral) or DMSO (0.06 %) in the absence or presence of NGF. PD-98059 did not affect Na^+ transport, but attenuated the NGF-induced reduction in Na^+ transport. These results indicate that NGF reduces Na^+ transport through a trkA-Erk1/2 mediated signaling pathway.

Key Words:
Ion transport, Nerve Growth Factor, Airway Epithelium, Lung, Electrophysiology

LESSENING CYTOTOXICITY USING NITROGEN-DOPING OF MULTI-WALLED CARBON NANOTUBES

Katelyn Siegrist[1], Steven H. Reynolds[1], Dale W. Porter[1], David Lowry[1], Michael L. Kashon[1], Michael McCawley[2], Jeffrey L. Salisbury[3], John Mastovich[4], Kristin Bunker[4], Mark Sparrow[4], Linda Sargent[1]

1National Institute for Occupational Safety and Health, Morgantown, WV, 2West Virginia University, School of Public Health, Morgantown, WV, 26506, 3Mayo Clinic, Rochester MN 55905, 4RJ LeeGroup, Monroeville, PA 15146

Multi-walled carbon nanotubes (MWCNT) have many unique applications in medicine, electronics and manufacturing. However, previous MWCNT exposure data have shown cellular necrosis, cell cycle disruption, chromosome errors and mitotic spindle aberrations in cultured immortalized human airway epithelial cells as well as increased necrosis and colony formation in primary human airway epithelial cells at concentrations anticipated in the workplace. The low density and small size of MWCNT, also makes respiratory exposures to workers likely during the production or use of commercial products. Combining the effects seen in vitro with the potential for lung deposition in the workplace MWCNT should be considered as presenting a potential health hazard to the exposed workers. On the other hand, nitrogen-doped MWCNT material has been shown to be less inflammatory in vivo than native MWCNT. In order to investigate the potential for lessened in vitro toxicity of nitrogen-doped MWCNT material we exposed immortalized and primary respiratory epithelial cells to 0.024, 0.24, 2.4, 24, and 48 µg/cm2 carbon nanotubes. Raman confocal analysis determined that both nitrogen-doped and native MWCNT material was taken up by the cell. Nitrogen-doped MWCNT material caused significantly less cellular necrosis 72 hours after exposure. Cell cycle analysis showed that, in both cell types, nitrogen-doped MWCNT did not cause a G1/S phase cell cycle block as previously seen with native MWCNT. This may have ramifications for lessening the toxicity of native MWCNT and protecting worker health. Research in progress is investigating the mitotic spindle disruption and chromosome errors following exposure to native and nitrogen-doped MWCNT at occupationally relevant doses.

DIFFERENTIAL GENE EXPRESSION IN SAEC AND HMVEC GROWN IN MONOCULTURE OR COCULTURE AND EXPOSED TO MWCNT: CORRELATION WITH *IN VIVO* STUDIES

Brandi N. Snyder-Talkington[1], Chunlin Dong[2], Xiangyi Zhao[2], Julian Dymacek[2,3], Dale W. Porter[1], Michael G. Wolfarth[1], Vincent Castranova[1], Nancy L. Guo[2], and Yong Qian

[1]Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV 26505

[2]Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV 26506

[3]Lane Department of Computer Science and Electrical Engineering, West Virginia University, Morgantown, WV 26506

The current interest in reducing and replacing the *in vivo* toxicity testing of nanomaterials, particularly multi-walled carbon nanotubes (MWCNT), in animals with *in vitro* cellular assays has led to the observation that *in vivo* and *in vitro* nanomaterial toxicity results are seldom concordant. As the majority of MWCNT *in vitro* toxicity testing is conducted in monoculture of a single cell type, the cellular communication and interactions inherent to the *in vivo* environment are lost. This study was conducted to determine the concordance between *in vitro* mRNA expression in monoculture and coculture cell cultures with *in vivo* mRNA expression in mouse lungs after MWCNT exposure. We exposed human small airway epithelial cells (SAEC) in monoculture, human microvascular endothelial cells (HMVEC) in monoculture, and SAEC and HMVEC grown together in coculture to 1.2 µg/mL MWCNT (Mitsui 7) for 6 and 24 h. Global mRNA profiling was conducted on monoculture and coculture cells and compared to global mRNA profiling in mouse lungs exposed to 0, 10, 20, 40, or 80 µg MWCNT (Mitsui 7) by aspiration for 1, 7, 28, or 56 days using a genome-wide correlation study. Overall coculture mRNA expression better correlated with overall mRNA expression from mouse lungs than did mRNA expression in either SAEC or HMVEC monoculture. Using a cutoff of 10% false discovery rate and 1.5 fold change, we determined that there were more concordant genes (gene expression both up- or downregulated *in vivo* and *in vitro*) in coculture than in monoculture. When reduced to only those genes involved in inflammation and fibrosis, known outcomes of *in vivo* MWCNT exposure, there were more disease-related concordant genes expressed in coculture than monoculture. As coculture gene expression better correlates with *in vivo* gene expression, we suggest that cellular cocultures may offer enhanced *in vitro* models for nanoparticle risk assessment.

GENERATIONAL EFFECTS OF GESTATIONAL ENGINEERED NANOMATERIAL EXPOSURE

PA Stapleton, CE Nichols, J Yi, VC Minarchick, JM Hollander, TR Nurkiewicz

Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV

As potential uses for engineered nanomaterials (ENM) continue to develop, so does the risk of inevitable exposure within non-occupational settings and non-traditional models, including expecting mothers and their unborn young. We have reported fetal and maternal effects from gestational ENM exposure; however, this study was designed to determine if gestational ENM exposure affects adult microvascular reactivity. Pregnant (gestation day 10) Sprague-Dawley rats were exposed to nano-titanium dioxide (TiO_2) aerosols with a count mode aerodynamic diameter of 144±8 nm, a concentration plateau of 11±0.3 mg/m^3, for 4 hrs/day, for an average of 5.5±0.5 days to evaluate the adult consequences of gestational ENM exposure. Overall, calculated cumulative maternal deposition over the exposure time was 85±3 µg. Dams delivered the litters normally and female adult progeny were between 8-12 weeks at the time of sacrifice. The arterioles of the heart and uterus (<150 µm) of the surviving adult female progeny were dissected, excised, and evaluated within an isolated microvessel preparation. Coronary microvascular responsiveness is impaired after gestational ENM exposure. This is represented by an impaired endothelium-dependent dilation to metabolic (acetylcholine, ACh, 10^{-9}-10^{-4} M), and mechanical stimuli (increased intraluminal flow, Flow, 5-30 µL/min), delayed smooth muscle relaxation (spermine-NONOate, 10^{-9}-10^{-4} M), and blunted α-adrenergic response of the smooth muscle (phenylephrine, 10^{-9}-10^{-4} M). Uterine arteriolar endothelial reactivity was also significantly impaired overall, presented as abolished endothelium-dependent reactivity (ACh), impaired flow induced dilation (Flow), and reduced myogenic responsiveness (transmural pressure, 15-120 mm Hg). We also evaluated isolated mitochondria of the left ventricle and uterine musculature. These studies suggest a reduction in respiratory control ratio within the left ventricle after gestational exposure, indicating mitochondrial dysfunction in both subsarcolemmal and intramyofibril subpopulations, which may have the propensity to increase oxidative stress production, thereby affecting NO bioavailability. Collectively, gestational ENM exposure may lead to independent cardiovascular consequences for the adult progeny of exposed mothers. These impairments may increase cardiovascular disease susceptibility and possible disturbances in conception for future generations.

NIH-F32-ES023435 (PAS)
AHA–13PRE16850066 (CEN)
NIH-RO1-ES015022 (TRN)
NSF-1003907 (VCM, TRN)
DGE-1144676 (VCM, CEN, TRN)

DIFFERENCES IN MWCNT FUNCTIONALIZATION INFLUENCE PRIMARY HUMAN LUNG EPITHELIAL CELL TRANSFORMATION POTENTIAL

Todd Stueckle[1,2], Donna Davidson[1], Raymond Derk[1], Michael Chen[2], Amruta Manke[2], Vince Castranova[1,2], Nick Wu[3], Yon Rojanasakul[2], Liying Wang[1,2]

[1] PPRB/HELD, NIOSH, Morgantown, WV 26505

[2] School of Pharmacy, West Virginia University, Morgantown, WV 26506

[3] Statler College of Engineering & Mineral Resources, West Virginia University, Morgantown, WV 26506

Multi-walled carbon nanotubes (MWCNT) have received increased scrutiny for potential long-term human health impacts based on their fibrogenicity and promotion of pulmonary carcinogenesis. Development of functionalized CNTs (fCNT) has intensified to improve surface activity in technological applications, and potentially reduce toxic effects. Sub-chronic *in vitro* CNT exposure causes neoplastic-like transformation; however, tumorigenic risk associated with fMWCNT exposure in human lung epithelium is presently unknown. To identify early steps in fCNT-induced cell transformation and CNT properties contributing to the effects, this study hypothesized that different surface functional groups on MWCNT determine their neoplastic transformation potential. Primary human small airway epithelial cells (SAECs) were continuously exposed (0.06µg/cm^2) to dispersed 'as prepared' (pMWCNT), carboxylated (cMWCNT), and aminated MWCNT (nMWCNT) for 8 and 12 weeks. Dispersed ultrafine carbon black (UFCB) and crocidolite asbestos served as particle controls. Exposed cells were assessed for several established cancer hallmarks and morphological transformation. UFCB, pMWCNT and nMWCNT cells exhibited increased mitochondrial activity at 48h while all MWCNT cells exhibited significant increased proliferation rates compared to controls. UFCB exposure stimulated significant invasion and migration behavior while pMWCNT and cMWCNT showed moderate significant increases; however, these trends disappeared at 6d post-exposure. Conversely, nMWCNT displayed the largest significant increase in colony formation potential while UFCB showed a moderate significant increase. All other treatments did not differ from controls. nMWCNT cells exhibited increased Type II foci frequency, indicative of cell transformation, compared to all other treatments. In summary, surface functionalization of carbon nanoparticles can impact transient, early neoplastic transformation events *in vitro* following occupationally relevant exposures.

Keywords: carbon nanotubes, cell transformation, functionalization, *in vitro* model, lung epithelial

Disclaimer: The findings and conclusions in this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

ACUTE ASPIRATION OF GRAPHENE SHEETS EVOKES AIRWAY HYPERREACTIVITY TO METHACHOLINE IN MICE

J.A. Thompson[1], J.S. Fedan[1], I.S. Chaudhuri[2], A. Kyrlidis[2], T. Sager[1], and J.R. Roberts[1]

[1]Pathology and Physiology Research Branch, NIOSH, Morgantown, WV 26505,

2Cabot Corporation, Billerica, MA 01821

Concern exists that the use of graphene sheets (GS) in composite materials might expose manufacturing workers to an inhalation hazard. Studies have shown that GS are cytotoxic in vitro (PC12 cells, fibroblasts) and in vivo in mice (lung granuloma), and Roberts et al. (2013) found non-oxidized GS with larger lateral dimensions (≥ 5 μm) and a greater number of layers (~20) produced more lung inflammation up to 7 d after aspiration in mice when compared to smaller GS (<1 μm laterally, ~ 4 layers). The lung toxicity of various forms of GS has not been characterized completely. Here, we investigated the effects of GS on basal lung resistance (RL), basal dynamic compliance (Cdyn), and reactivity to inhaled methacholine (MCh) aerosol. Mice were given a non-oxidized GS (5 μm x 5 μm laterally, 7 nm thick equal to ~ 20 layers; 40 μg) suspended in dispersion medium (DM; Porter et al., 2008) or DM (control) via aspiration. RL and Cdyn reactivity to increasing concentrations of MCh aerosol were measured 4 h - 2 mo after GS exposure. Basal RL was increased and Cdyn was decreased at 1 d post-exposure but at no other time. Airway reactivity to MCh (as %ΔRL, %ΔCdyn) was increased at 4 h post-exposure, but it diminished at 1 d and 7 d post exposure. GS was essentially without effect on RL or Cdyn at 1 mo and 2 mo after administration. The results indicate that a single exposure to GS increases transiently lung resistance and reactivity to MCh.

ISOLATION METHODS CAUSE VARIANCES IN EXOSOMAL PROTEIN CONTENT: IMPLICATIONS FOR BIOMARKER DISCOVERY

Lauren M. Wagner[1], Justin Hettick[1], Diane Schwegler-Berry[2], Angela Lemons[1], Toni Bledsoe[1], Paul D. Siegel[1]

[1]Allergy and Clinical Immunology Branch, Health Effects Laboratory Division, National Institute of Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV. [2]Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute of Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV.

Exosomes are vesicles that are released by most cell types and are present in most bodily fluids. Under immunological stimulation the number of exosomes released increases, indicating that exosomes may carry markers specific to stimulation. Specific RNAs and proteins are targeted into exosomes and consequently, exosomal protein and RNA content differ from that of the secreting cell. Based on these findings, exosomes have received significant attention as carriers of biomarkers. Currently, the accepted method for exosome isolation involves ultracentrifugation, which is time consuming and results in low yields. Several commercial products have become available that boast reduced preparation time and higher yields. The goal of this work was to compare the protein content and purity of exosomes isolated using commercial kits to that of ultracentrifugation. Exosomes were isolated from human serum using four methods: i. ExoQuick kit (System Biosciences, Mountain View, CA), ii. ExoQuick kit followed by dialysis (300 kDa cutoff), iii. ExoSpin kit (Cell Guidance, Carlsbad, CA), and iv. ultracentrifugation. After isolation, exosomes were characterized using electron microscopy and gel electrophoresis, and exosomal proteins were identified using mass spectrometry. Exosomes isolated from commercial kits had appreciable levels of non-exosomal protein contaminates compared to ultracentrifugation. The ExoQuick kit combined with dialysis reduced this contamination to levels observed with ultracentrifugation. For biomarker discovery, protein contamination is undesirable as the contaminating proteins can mask the presence of less abundant, but more relevant molecules and can contribute to apparent yield, which is frequently measured by total protein content. Here we present a novel approach using the ExoQuick purification kit combined with dialysis that reduces isolation time while increasing the purity of isolated exosomes for application in biomarker discovery.

BUTTER FLAVORING-ELICITED RELAXATION AND BIOELECTRIC RESPONSES OF RAT AIRWAY ARE INDEPENDENT OF BITTER TASTE RECEPTORS (TAS2R)

E.J. Zaccone[1], M.J. Shimko[1], J.A. Thompson[2], and J.S. Fedan[2]

[1]Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, WV 26506 and [2]Pathology and Physiology Research Branch, NIOSH, Morgantown, WV 26505

"Popcorn workers' lung" is a fixed obstructive pulmonary disease caused by inhalation of artificial butter flavoring vapor. In the isolated, perfused rat trachea preparation, the butter flavorings, diacetyl and 2,3-pentanedione elicit airway smooth muscle (ASM) relaxation. TAS2Rs mediate relaxation of ASM by increasing the concentration of intracellular Ca^{2+} via large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels. This study investigated the potential involvement of TAS2Rs in relaxant responses of tracheal ASM and bioelectric responses of tracheal epithelial cells in response to denatonium (1 mM), a TAS2R agonist, diacetyl (3 mM) and 2,3-pentanedione (3 mM). In rat tracheal strips contracted with methacholine (3×10^{-5} M), denatonium-induced relaxation responses were inhibited by iberiotoxin (IbTx; 100 nM), a BK_{Ca} channel blocker, suggesting that denatonium-induced relaxation is mediated by TAS2R. However, relaxation responses to the butter flavorings were not antagonized by IbTx. Whether TAS2R mediate bioelectric responses to denatonium and flavorings in the epithelium was investigated. The decrease in short-circuit current (I_{sc}) in response to denatonium and flavorings were not blocked by apical IbTx, suggesting that the responses in the epithelium were not mediated by TAS2R. Our findings indicate that BK_{Ca} channels are not involved in flavoring-induced bronchodilation or I_{sc} responses in rat airways.

EFFECTS OF BUTTER FLAVORING VAPOR EXPOSURE ON CULTURED PRIMARY HUMAN TRACHEAL/BRONCHIAL EPITHELIAL (HTBE) CELL ION TRANSPORT

E.J. Zaccone[1], W.T. Goldsmith[2], M.J. Shimko[1], J.R. Wells[2], J.A. Thompson[2], and J.S. Fedan[2]

[1]Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, WV 26506 and [2]Pathology and Physiology Research Branch, NIOSH, Morgantown, WV 26505

In the microwave popcorn industry, inhalation of butter flavoring may result in “popcorn workers’ lung,” a disease resembling bronchiolitis obliterans. Rats exposed for 6 h to vapor from butter flavorings diacetyl and 2,3-pentanedione demonstrate flavoring concentration-dependent damage of the upper airway epithelium. Because epithelial ion transport is essential for maintenance of transcellular electric potential, fluid transport and cellular volume, we investigated the effects of flavoring vapor exposure on ion transport in HTBE cells. We hypothesized that epithelial ion transport may be among the earliest targets associated with the onset of butter flavoring toxicity. Using a novel exposure system designed for cultured cells, HTBE cells were exposed for 6 h to diacetyl or 2,3-pentanedione vapors (25 ppm or >60 ppm). After exposure, cells were placed in an Ussing system to record short-circuit current (I_{sc}) at the 0 h or 18 h time-point. Flavoring (25 ppm) exposure reduced amiloride (apical; 3.5×10^{-5} M)-sensitive Na^+ transport as compared to controls. This reduction in Na^+-dependent I_{sc} was not accompanied by changes in transepithelial resistance and recovered 18 h after exposure. Concentrations of diacetyl and 2,3-pentanedione above 60 ppm resulted in cell death. Our results demonstrate that flavorings reduce apical Na^+ conductance in airway epithelium.

Allegheny - Erie SOT 2014 SPONSORS

A-E SOT is extremely fortunate to enjoy the generous support of our sponsors. This meeting would not be possible without the significant commitments made by these groups. Words cannot fully convey our gratitude for all the 2014 contributions. We hope that the increased impact of this meeting, Chapter growth and the continued development of our relationships justify their investments both professionally and personally.

GOLD ($1500 +)

	Many thanks are given to the WVU Department of Physiology and Pharmacology for their continued and generous support of the Allegheny-Erie Society of Toxicology Meeting.
	RJ Lee Group specializes in the characterization of nanomaterials from analysis of nanoparticles to evaluation and testing of products incorporating nanomaterials. RJ Lee Group also works with the nanotechnology community by providing consulting services related to potential environmental health and safety (ESH) issues involving the use of nanomaterials in research activities and commercial products.
	Myriad RBM, Inc. is the world's leading multiplexed immunoassay testing laboratory, providing comprehensive protein biomarker services based on its Multi-Analyte Profiling (MAP) technology platform. This platform provides pre-clinical and clinical researchers with reproducible and quantitative data for a few or hundreds of proteins in a cost-effective manner. To learn more, visit www.myriadrbm.com

SILVER ($1000-1500)

INGENUITY® SYSTEMS	SOT | Society of Toxicology Creating a Safer and Healthier World by Advancing the Science of Toxicology

BRONZE (up to $1000)

WIL RESEARCH	ThermoFisher SCIENTIFIC
DSI™	CCRS Center for Cardiovascular and Respiratory Sciences

Allegheny-Erie Society of Toxicology - Regional Chapter

2014-2015 Officers

President:	Patti Zeidler-Erdely, Ph.D.	paz9@cdc.gov
President- Elect:	Phoebe Stapleton, Ph.D.	pstapleton@hsc.wvu.edu
Vice President:	Aaron Barchowsky, Ph.D.	aab20@pitt.edu
Secretary:	Robin Gandley, Ph.D.	rgandley@mwri.magee.edu
Treasurer:	William Mackay, Ph.D.	wmackay@edinboro.edu
Councilors:	Kelly Brant, Ph.D.	kab124@pitt.edu
	James Fabisiak, Ph.D.	fabs@pitt.edu
	Elaine Freeman, Ph.D.	efreeman@exponent.com
	Jim Scabilloni, Ph.D.	zbc9@cdc.gov
	Timothy Nurkiewicz, Ph.D.	tnurkiewicz@hsc.wvu.edu
	Mark Weisberg, M.S.	Mark.Weisberg@cbifederalservices.com
Post-Doctoral Representative:	Kevin Beezhold, Ph.D.	beezhold@pitt.edu
Graduate Student Representative:	Cody Nichols	cenichols@hsc.wvu.edu
K-12 Outreach Representative:		
Past President:	Aaron Erdely, Ph.D.	efi4@cdc.gov

A-E SOT Website: http://www.toxicology.org/isot/rc/allegheny


A-E
SOT