ALLEGHENY-ERIE REGIONAL CHAPTER
of the
SOCIETY OF TOXICOLOGY

ANNUAL REPORT: 2014-2015

May 1, 2014-April 30, 2015

I. Officers:

Office	2014-2015	2015-2016
President:	Patti C. Erdely	Phoebe Stapleton
President-Elect:	Phoebe Stapleton	Jim Antonini
Vice President:	Aaron Barchowsky	Vince Castranova
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Councilors:	Mark Weisberg	Mark Weisberg
	Jim Scabilloni	Jim Scabilloni
	James P. Fabisiak	James P. Fabisiak
	Tim Nurkiewicz	Tim Nurkiewicz
	Kelly A. Brant	Todd Stuekle
	Elaine Freeman	Elaine Freeman
PDA Representative:	Kevin Beezhold	Kevin Beezhold
GSLC Representative:	Cody Nichols	Cody Nichols
K-12 Outreach:	William J. Mackay	William J. Mackay

President's comment: The last year was another very successful for A-E SOT. This is directly attributable to the energy and commitment of the current officers. 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, Jim Antonini, Phoebe Stapleton
Web:	Jim Scabilloni
RC4 Representative:	Todd Stueckle

III. Activities:

a) 2015 A-E SOT Annual Meeting

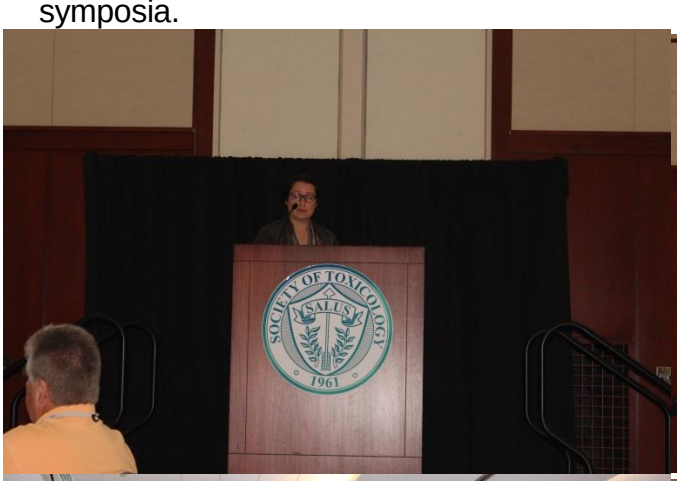
Location / Date: Erickson Alumni Center, Morgantown, West Virginia. June 3rd and 4th, 2015.
Pre-Registered Attendees: 70

Highlights:

1) The 3 symposia during the 2-day conference were headlined by the invited speakers: Dr. Ralph Cooper (top left), Dr. Andrew Ottens (top right), Dr. Ken Hastings (bottom left), Dr. Raymond David (bottom right), and Dr. Aaron Erdely who each presented in one of the scientific sessions.

2) West Virginia University President, Dr. E. Gordon Gee, generously provided attendees with a welcome address, highlighting the importance of the field and the contributions being made to the field of toxicology at WVU. Please note Dr. Gee's address (below left) and meeting with Dr. Stapleton (current AE-SOT President) and Dr. Timothy Nurkiewicz (Current AE-SOT Councilor and Past-President). Another aspect of Dr. Gee's address indicated the ongoing support from WVU for A-E SOT endeavors.

3) Two young investigators (Dr. Jen Gallager, top left), four post-doctoral fellows (Dr. Stephanie Rellick, top right; Dr. Donna Davidson, bottom left), four graduate students (Mr. Vincent Nyakubaya, bottom right), and one of our sponsors (Myriad/Rules Based Medicine) also presented in these symposia.



4) Following our first day and two scientific sessions we held a poster session over refreshments in conjunction with a workshop and demonstration from one of our vendors (DMT). Overall, there were 28 attendee and 3 exhibitor poster presentations. Please see the supplemental program for the scientific abstracts. Posters and presentations were judged by Dr. Andrew Ottens (invited speaker) and Dr. Travis Knuckles (A-E SOT member).

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 was maintained from the previous years with a total of 16 trainees and 11 mentors participating. We consider this to be one of our most successful endeavors. We were grateful for the generous support from the SOT Career Resource and Development (CRAD) committee to make this luncheon for our trainees possible.

6) 2014 A-E SOT Awards:

2014 Maryanne Stock Student Research Award
Samantha Adkins; Mentor: Dr. Brian Antonsen
Affiliation: Marshall University
Project: *The Effects of Silver Nanoparticles on Crayfish Synaptic Transmission*

2015 Graduate Student Travel Award
Amin Cheikhi
University of Pittsburgh Graduate School of Public Health
Mentor: Dr. Aaron Barchowsky

2015 Graduate Student Travel Award
Carrie Long
West Virginia University – CDC-NIOSH
Mentor: Dr. Stacy Anderson

2015 Postdoctoral Fellow Travel Award
Dr. Melissa Badding
West Virginia University – CDC-NIOSH
Mentor: Dr. Steve Leonard

2015 Best Graduate Student Poster Presentation
Lindsey Bishop
West Virginia University – CDC-NIOSH
Abstract: *The Effect of Polymer Coatings in the Toxicity of Multi-Walled Carbon Nanotubes*
Mentor: Dr. Aaron Erdely

2015 Best Graduate Student Oral Presentation
Sharlee Mahoney
University of Pittsburgh Department of Chemical Engineering
Abstract: *Structure-Toxicity Correlation for Complex Engineered Ni/SiO_2 Nanomaterials Using High-Throughput Zebrafish Assays*
Mentor: Dr. Veser

2015 Best Postdoctoral Fellow Poster Presentation
Dr. Colleen McLoughlin
National Institute for Occupational Safety and Health
Abstract: *Lung Toxicity and Allergy Responses in Mice Exposed to Nanoparticle Silver*
Mentor: Dr. Jenny Roberts

2015 Best Postdoctoral Fellow Oral Presentation
Dr. Donna Davidson
National Institute for Occupational Safety and Health
Abstract*: Fibrogenic Potential of Nano-scaled Cerium Dioxide is Affected by Physiochemical Property Changes*

2015 Best Methodology
Carrie Long
National Institute for Occupational Safety and Health and West Virginia University
Abstract: *Immunoregulatory Potential of MicroRNA 210 in a Murine Model of Chemical Sensitization*
Mentor: Dr. Stacy Anderson

2015 Best Overall Presentation
Dr. Stephanie Rellick
West Virginia University
Abstract: *Evaluation of Hydraulic Fracturing Chemicals on Neuronal Cell Mitochondria Function*

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 (University of Pittsburgh, Edinboro University of Pennsylvania, and Indiana University of Pennsylvania) to attend both days of the annual meeting.

In the past these students were unable to attend both days due to lack of funds for overnight lodging.

8) Following our poster session on Day 1, our Chapter was able to host a small informal reception for all attendees. This was held to further increase interaction between our invited speakers and meeting attendees.

9) Lastly, the 2015 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 2015 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 if Council is interested.***

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. This has worked well thus far, although can be difficult during registration for our non-SOT member guests.

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).

VI. 2016 Annual Meeting: We have tentatively selected May 2016 for next year's meeting. This will be held again at the WVU Erikson Alumni Center (http://alumni.wvu.edu/eac). Given the welcome from Dr. Gee this past year, in addition to the financial and administrative support already committed by WVU Department of Physiology & Pharmacology and the WVU Center for Cardiovascular & Respiratory Center for 2016. Similar to our well-sponsored meetings in 2014 and 2015, we plan to secure necessary 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:

- At the National SOT meeting in San Diego, A-E SOT again 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. This is the third joint meeting for the chapters and by far the largest. At the reception, we ran out of food and drink tickets so quickly we had to order another round of food to keep up with demand! This unanticipated expense pushed our chapter over our reception budget, but given the significant turn out from both chapters, it was the best decision for the attendees from both chapters. A few images from the reception are below with all EC attendees from both chapters on the next page.

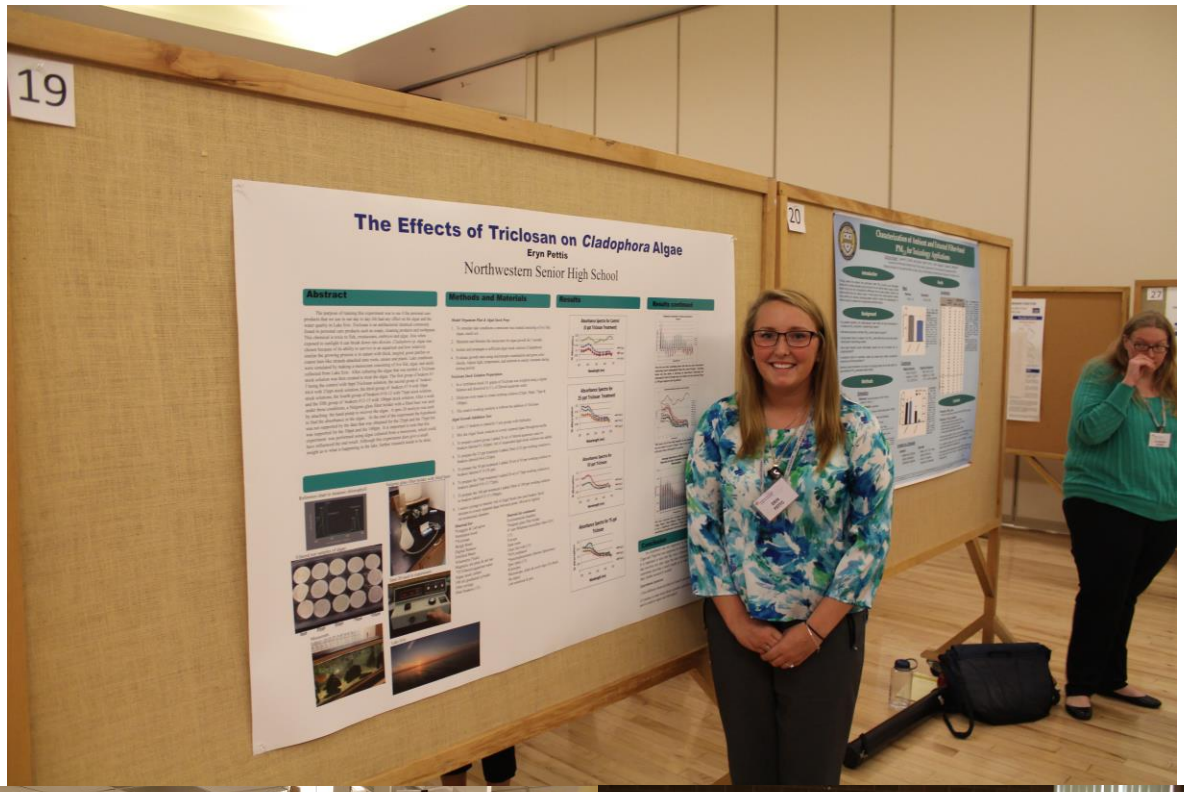
Photos from the Michigan Chapter Joint Reception




VII. Budget/Bank Statement: Generous donations from sponsors (The WVU Department of Physiology & Pharmacology, the WVU Center for Cardiovascular & Respiratory Center, WV NanoSAFE, Myriad / RBM, Qiagen, Ingenuity Systems, RJ Lee Group, Protea, DMT, and DSI) and an increase in meeting attendance resulted in ~$27,429 (June financial statement) in the A-E SOT account at the time of this report, a nearly $1500 increase from last year at this time. When all sponsors are accounted for and balances have been paid, the chapter will have increased their account by ~$4500 compared to 2013. Furthermore, we are now operating in the black for four 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, 3) continue our joint reception with the Michigan Regional Chapter at the Annual meeting and 4) 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. For 2015-2016, William Mackay will lead our K-12 outreach. Several new avenues have been discussed. 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. Outreach is a goal of our

Chapter for the 2015-2016 year; we have had small successes with laboratory shadowing (2 area HS students [Miss Sarah Jones and Miss Hannah Reed] and 1 HS teacher [Mr. Kurt. Jones] all from West Greene County HS, PA), classroom presentations (University HS, Morgantown, WV), and summer internship of a regional HS teacher (Miss Samantha Simon, University HS, Morgantown, WV). We are hoping that these initial contacts and additional support from SOT will enhance these activities at/with HS in our regional area and pave the way for future outreach. Please see below, Miss Eryn Pettis presenting her work at our Chapter meeting (below) and please find her abstract in the attached program, Miss Sarah Jones using intravital microscopy to explore the microcirculation (bottom left) and Miss Samantha Simon presenting her summer work with one of her mentors (Dr. Stapleton) at the WV-INBRE conference (bottom right).



VIV. Other: We are planning administrative changes in the 2015-2016 year to amend the bylaws to add an additional graduate student and postdoctoral representative. With the growth of the chapter and the growth of our Lunch with and Expert and Joint Regional Chapter (both organized in-part by these representatives) the burden and expectation on these individuals is great, largely due to the geographic make-up and unique professional opportunities in our Region. For example, during a recent A-E SOT meeting, the students were able to tour the National Institute for Occupational Safety and Health, an opportunity set up by a student primarily working in Morgantown. This was a greatly enjoyed tour for our Pittsburgh colleagues, who would not have been able to coordinate on their own, without the local contact. Therefore, we will be looking to add additional representation in these positions, one in Pittsburgh and one in Morgantown. With this addition will come an added cost of travel support for these trainees to attend the national meeting, increasing our financial need as we support travel for 4 representatives.

29th Annual Meeting of the Allegheny-Erie Society of Toxicology Regional Chapter

Erickson Alumni Center
West Virginia University
Morgantown, WV
June 3-4, 2015

SCHEDULE

Wednesday, June 3

9:00–11:00 Registration
Lunch

10:45–11:00 Welcome and Announcements

***SYMPOSIUM 1*:**

11:00–1:30 **Neuroendocrine Toxicology**
- 11:00 Introduction/Symposium Overview (moderator - James Simpkins, Ph.D.)
- 11:05 Keynote speaker - Ralph Cooper, Ph.D.; U.S. EPA
- 12:05 Vincent Nyakubaya; West Virginia University
- 12:25 Stephanie Rellick, Ph.D.; West Virginia University
- 12:45 Shaina Stacy, Ph.D.; University of Pittsburgh
- 1:05 Invited speaker - Andrew Ottens, Ph.D.; Virginia Commonwealth University

1:30–2:00 - Welcoming Remarks - E. Gordon Gee, President, West Virginia University
Break

***SYMPOSIUM 2*:**

2:00–4:30 **Immunotoxicology**
- 2:00 Introduction/Symposium Overview (moderator – Ralph Cooper, Ph.D.)
- 2:05 Keynote speaker - Ken Hastings, Dr.P.H., DABT, Fellow ATS; Hastings Toxicology Consulting
- 3:05 Carrie Long; West Virginia University/NIOSH
- 3:25 Ajay Nayak, Ph.D.; NIOSH
- 3:45 Rich Stratton; Myriad/Rules Based Medicine
- 4:05 Nikki Marshall, Ph.D.; NIOSH

4:30–6:00 Poster Session – Networking – Refreshments
DMT workshop/demo

6:00 Announcements and Adjourn (Group Dinner-location to be determined)

Thursday, June 4

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

***SYMPOSIUM 3*:**

9:00–11:50 **Nanotoxicology**

9:00 Introduction/Symposium Overview (moderator – Vince Castranova, Ph.D.)
9:05 Keynote speaker - Raymond David, Ph.D.; BASF Corporation
10:05 Katie Dunnick; West Virginia University/NIOSH
10:25 Sharlee Mahoney; University of Pittsburgh
10:45 Donna Davidson, Ph.D.; NIOSH
11:05 Jennifer Gallagher, Ph.D.; West Virginia University
11:25 Aaron Erdely, Ph.D.; NIOSH

11:50–12:45 "Lunch with an Expert" & Networking sponsored by QIAGEN/Ingenuity and SOT CRAD DMT workshop/demo

12:45–1:15 Awards

1:15 Closing Comments and Adjourn

SPEAKER BIOGRAPHIES

1) Ralph Cooper, Ph.D.

United States Environmental Protection Agency
Research Triangle Park, North Carolina

Ralph Cooper received his Ph.D. in Psychobiology from Rutgers University in 1973 and postdoctoral training in the Duke University Neurosciences Program and the Center for the Study of Aging and Human Development. In 1976, he joined the Duke University Medical School faculty with appointments in Psychiatry and Psychology conducting research on the causes and consequences of age-dependent changes in brain chemistry. He joined the staff at the National Health and Ecological Effects Research Laboratory, (NHEERL), U.S. EPA in 1984 where he served as a research biologist, and held positions as Chief of the Endocrinology/Gerontology Section and Chief of the Endocrinology Branch, Reproductive Toxicology Division. Dr. Cooper has served on several study sections for NIEHS and U.S. EPA, and the editorial board of Editorial Board of Experimental Aging Research, the Neurobiology of Aging and Birth Defects Research: Developmental and Reproductive Toxicology. He is an adjunct professor at Duke University, Department of Psychology and the North Carolina State School of Veterinary Medicine. While at NHEERL, he maintained an active research program examining the effect of environmental chemicals on neuroendocrine function. He has over 150 publications in this area, the results of which have had a major influence on regulatory decisions by the Agency. Two of his papers were voted the “Best Paper Published in Toxicological Sciences” by the Reproductive and Developmental Toxicology Specialty Section of the Society of Toxicology. Dr. Cooper has participated in a number of critical activities for EPA such as developing the background material and presenting at a number of Science Advisory Panels dealing with critical issues on the regulation of environmental chemicals. He and members of the Endocrinology Branch played a significant role in the development, validation and implementation of many of the Tier 1 test included in the Agency’s Endocrine Disruptor Screening and Testing Program. He is the recipient of several awards from the Office of Research and Development, Office of Pesticide Programs and NHEERL for his contributions to the Agency including a Gold Medal for exceptional service, Silver and Bronze Medals recognizing the impact of his programmatic contributions and several Scientific and Technical Achievement Awards. He is a founding member of the Triangle Consortium for Reproductive Biology, an organization that promotes communication and information sharing among North Carolina colleges, universities and government institutions. At the international level, Dr. Cooper served as co-chair of the International ISLI/HESI Agricultural Chemical Safety Assessment Committee which re-evaluated the regulatory tests required for protecting human health. He contributed significantly to the development of new guidelines for multigenerational test for the Organization of Economic Cooperation and Development (OECD). He has also served on expert workgroups and panels with the World Health Organization’s International Programme on Chemical Safety, and the World Trade Organization addressing issues related to the impact of chemical exposure on human health.

2) Kenneth L. Hastings, Dr.P.H., DABT, Fellow ATS

Hastings Toxicology Consulting LLC
Mount Airy, Maryland

Dr. Hastings received his Doctor of Public Health degree from the School of Public Health, University of North Carolina at Chapel Hill, in 1987. He completed his doctoral research at the Chemical Industry Institute of Toxicology under the direction of Dr. Jack Dean. Dr. Hastings then served as a US Peace Corps Volunteer in Fiji under the Permanent Secretary for Health. From 1988 – 1991 Dr. Hastings completed a post-doctoral fellowship and was then appointed Research Associate in the Pharmacology/Toxicology Research Laboratory, Department of Anesthesiology, College of Medicine, University of Arizona, under the direction of Dr. A. Jay Gandolfi. From 1991 – 2007 Dr. Hastings worked in the Center for Drug Evaluation and Research, US Food and Drug Administration, in various positions: pharmacology/toxicology reviewer in the Division of Anti-viral Drug Products (1991 – 1997), pharmacology/toxicology supervisor in the Division of Special Pathogen and Immunologic Drug Products (1997 – 2003), and as an Associate Director in the Office of New Drugs (2003 – 2007). Dr. Hastings served on numerous committees and working groups while at FDA, including lead representative for CDER/FDA on the ICCVAM validation of the murine local lymph node assay and as lead negotiator on ICH S8 (Immunotoxicology Evaluation of New Drugs). From 2007 – 2014 Dr. Hastings was Associate Vice President for Regulatory Policy, Sanofi US, serving as corporate representative on numerous industry working groups dealing with regulatory science and policy. Dr. Hastings has served in many professional societies (President, Society of Toxicology Immunotoxicology Specialty Section; President, American College of Toxicology; President, SOT Regulatory and Safety Evaluation SS). Dr. Hastings served as President of the American Board of Toxicology and has numerous publications, included editor of books on use of mini-pigs in biomedical research and international standards for nonclinical safety evaluation of medical products. Dr. Hastings is currently an independent consultant specializing in toxicology studies in drug development.

3) Raymond David, Ph.D.

BASF Corporation
Florham Park, New Jersey

Dr. Raymond David is Manager of Toxicology for Industrial Chemicals in BASF Corporation. He received his Ph.D. in Pharmacology from the University of Louisville, after which he was a Postdoctoral Fellow at the Chemical Institute of Toxicology in Research Triangle Park. Dr. David worked for 8 years at Microbiological Associates in Bethesda, Maryland where he managed the Inhalation and Mammalian Toxicology Departments. He also spent 14 years at Eastman Kodak in Rochester New York as Senior Toxicologist before joining BASF in 2006. Dr. David has experience conducting inhalation, pulmonary, reproductive, and systemic toxicity studies. He led a team responsible for EH&S issues for nanotechnology at Eastman Kodak Company, and currently is responsible for nanotechnology issues in BASF Corporation. Dr. David is the BASF Corp representative on the Nanotechnology Panel of the American Chemistry Council, and he participates in the US Technical Advisory Group to ISO TC 229 activity on nanotechnology.

4) Andrew Ottens, Ph.D.

Assistant Professor
Anatomy and Neurobiology
Virginia Commonwealth University School of Medicine, Richmond, VA

Dr. Ottens is interested in the systems biology underlying neurobiological health impacts of inhalation toxicity. His research aims to understanding the mechanisms by which inhalation of xenobiotic agents may alter neurobiology and induce neurological deficits. Employing omic approaches, Dr. Ottens' group is investigating the neuropathogenic role of induced by-products released into circulation following pulmonary insults. Further, his laboratory is interested in how inhalation exposures can influence bioenergetics and synaptic connectivity within circuits related to induced functional deficits. The long-range goals of these studies are to substantiate neurobiological consequences induced by inhaled pollutants, understand the mechanisms of action, and devise translatable diagnostics with which to assess at-risk human populations.

ORAL PRESENTATION ABSTRACTS

A NEW ANALYTICAL TECHNIQUE TO PROFILE MULTIPLE STEROIDS IN INDIVIDUAL FEMALE ZEBRAFISH TO SHED LIGHT ON MECHANISMS OF ENDOCRINE DISRUPTION

VT Nyakubaya,[1] BC Durney,[1] MCG Ellington,[1] AD Kantes,[1] PR Reed,[1] SE Walter,[1] J Ripley-Stueckle,[2] LA Holland[1]

[1]C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV
[2]C. Department of Biology, West Virginia University, Morgantown, WV

Exposure to endocrine disrupting chemicals is associated with reproductive impairment as well as health issues including cancer, diabetes, and obesity. A new capillary electrophoresis method has been developed and applied to analyze circulating steroids in plasma volumes less than 5 microliters. This research is significant because it is enables rapid analysis of multiple circulating steroids, which generates more information about the mechanism of endocrine disruption. This is innovative because for the first time multiple steroids are analyzed in limited volume plasma samples from single fish. The capillary electrophoresis method can analyze 5 natural steroids in 5 minutes from ≤ 5 microliters of plasma collected from a single zebrafish. With a technique called pH-mediated stacking, limits of detection ranging from 0.2 to 2 ng/mL (0.8 to 6 nM) for the steroids are achieved with ultraviolet-visible absorbance detection. This steroid assay was utilized to assess circulating steroids in zebrafish exposed to 17β-estradiol, a positive control for estrogenic activity, using protocol outlined by the Organisation for Economic Co-operation and Development Test No. 229: Fish Short Term Reproduction Assay. The reproductive success was evaluated through egg production and hatching. Plasma analysis revealed that exposure to 17β-estradiol leads to an increase in the level of circulating estrone in female zebrafish. Estrone is produced from 17β-estradiol in the steroid synthetic pathway. Thus, by monitoring the circulating steroids using this new technique, more information is obtained about the mechanism of disruption. An increase in the level of circulating estrone is also observed in fish that has been exposed to acetone, which is used as a delivery solvent for toxicity studies. Therefore, care must be taken when choosing the solvent vehicle to be used to expose the fish to the endocrine disrupting chemical. The use of capillary electrophoresis in endocrine disruption toxicity study is a fast method that gives insight into the mechanism of disruption in individual fish.

EVALUATION OF HYDRAULIC FRACTURING CHEMICALS ON NEURONAL CELL MITOCHONDRIAL FUNCTION

SL Rellick [1] and JW Simpkins [2]

[1] Department of Physiology and Pharmacology, WVU School of Medicine, Morgantown, WV
[2] WVU School of Medicine, Morgantown, WV

Hydraulic fracturing is a method used to extract natural gas buried beneath natural barriers. The chemical additives used in hydraulic fracturing serve different purposes, such as preventing microbial growth, preventing pipe corrosion, reducing friction and dissolving minerals to create fractures in the rock. The chemical additives included in this study are N,N-dimethylformamide, glutaraldehyde and acrylamide, as a component of polyacrylamide. The objective of these studies are to investigate compounds commonly used in hydraulic fracturing to determine their effects on neuronal cell viability and mitochondrial function. Mitochondrial health, as measured by efficiency of oxidative phosphorylation (OxPhos) and reactive oxygen species (ROS) production, determine neuronal viability. To evaluate toxicity, we exposed HT-22 hippocampal neurons to acrylamide, DMF or glutaraldehyde and completed a Calcein AM assay. To investigate mitochondrial function, we exposed HT-22 cells the fracking chemicals and completed a Mito-Stress Test using the XFe96 Bioanalyzer. Finally, we exposed cells to the fracking chemicals and evaluated ROS production using H2-DCFDA. Acrylamide (1 ppm) and glutaraldehyde (100 ppb and 1 ppm) increased cell death. DMF did not cause cell death at any of the tested concentrations. Interestingly, the lowest concentrations of fracking chemicals increased mitochondrial function in the Mito-Stress Test assay, suggesting that low exposure to these chemicals induces a biological response. The increase in mitochondrial activity could not be explained by increased ROS production, as ROS was not significantly increased in response to these chemicals. Residents living in drilling-dense regions have the potential for chronic, low dose exposure to chemical additives, and it is important to evaluate the health implications of exposure to chemicals used in hydraulic fracturing especially since many of the chemical additives have known toxicity in humans.

Funding: JWS Start-Up Funds (WVU), NIH P01 AG022550 and NIH P01 AG027956

INVESTIGATING PRENATAL EXPOSURE TO GROUPS OF AIR TOXICS AND AUTISM SPECTRUM DISORDER USING EXPLORATORY FACTOR ANALYSIS

SL Stacy[1] and EO Talbott[2]

[1]Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA
[2]Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interaction and rigid behaviors and routines. The prevalence of ASD has increased markedly over the last several decades, motivating investigations into possible causes and risk factors. One area of interest has been the role of the environment, particularly ambient air pollution, in the development of childhood ASD. However, few studies have explored groups of air toxics, such as those emitted from a common source, in relation to ASD risk. In the present study, estimates of 30 ambient air toxics from the 2005 National Air Toxics Assessment (NATA), modeled at the census tract level, were linked to 217 cases of ASD and 224 controls born in southwestern Pennsylvania from 2005 to 2009. An exploratory factor analysis (varimax rotation) was conducted to reduce these 30 pollutants to a set of key predictors (factors). Factor scores were calculated using two methods: index scores based on sums of quartiles of exposure and linear regression. These scores informed two sets of logistic regression models to determine which factors were associated with increased ASD risk, unadjusted and adjusted for mother's age, race, education, and smoking. The results of each method for calculating factor scores were compared. A Spearman correlation matrix revealed that many of the 30 NATA air toxics were highly correlated with each other. The air toxics loaded onto 7 main factors. Regardless of the method used to calculate the scores, the factors most associated with increased ASD risk appeared to represent traffic, other combustion sources, and certain types of manufacturing (plastics, rubbers, and adhesives). These sources should be targeted in future investigations of ASD risk and air pollution.

IMMUNOREGULATORY POTENTIAL OF MICRORNA 210 IN A MURINE MODEL OF CHEMICAL SENSITIZATION

CM Long[1, 2], NB Marshall[2], E Lukomska[2], AP Nayak[2], PD Siegel[2], BJ Meade[2], SE Anderson[2]

[1]Immunology and Microbial Pathogenesis Graduate Program, WVU, Morgantown, WV
[2]CDC-NIOSH, Morgantown, WV

MicroRNAs are functionally significant, single-stranded RNAs that regulate gene expression; however, their roles in chemical sensitization have not been elucidated. Toluene 2,4-diisocyanate (TDI) is a low molecular weight chemical sensitizer that is a significant cause of occupational asthma. In order to investigate the functional role of a microRNA that is upregulated during TDI sensitization (miR-210), BALB/c mice were dermally exposed to TDI (0.5-4% v/v) and gene and protein expression were evaluated in the draining lymph nodes (dLN) and ears using RT-PCR and Western blot/flow cytometry, respectively. Sensitization was confirmed by increased total serum IgE levels. Augmented miR-210 expression was observed in the dLN and ears following exposure to both irritating and non-irritating concentrations of TDI. Increased expression of a miR-210 inducer, *hif1α*, was observed in the ears and dLN at various time points following TDI exposure. Alterations in expression of confirmed miR-210 transcription factor target foxp3 were observed in the dLN and ears and decreases in predicted targets' (foxp3, runx1, runx3, and smad4) mRNA expression were observed in the dLN of TDI-exposed mice. These transcription factors are directly involved in regulatory T cell (T_{reg}) differentiation and function; therefore, miR-210 may play a functional role in TDI sensitization by potentially detrimentally affecting T_{reg} development and functional capacity. This hypothesis is supported by the T_{reg} population's kinetics and the presence of miR-210 in $CD4^+$ T cells during TDI sensitization. Because the roles of T_{regs} and miRNAs in chemical sensitization have not been elucidated these data contribute critically needed insight into the immunologic mechanisms of chemical-induced allergic disease and are critical for the development of preventative and therapeutic strategies.

IMMUNOPATHOLOGICAL OUTCOMES FOLLOWING SUBCHRONIC ASPERGILLUS FUMIGATUS EXPOSURE IN TWO DIVERSE MOUSE STRAINS

AP Nayak[1], BJ Green[1], AR Lemons[1], TL Croston[1], WT Goldsmith[1], DR Germolec[2], DH Beezhold[1]

[1]Health Effects Laboratory Division, NIOSH, Morgantown, WV 26505
[2]National Toxicology Program Division, NIEHS, Research Triangle Park, NC 27709

Fungi are widely known to contaminate building materials and agricultural commodities. Personal exposure to fungal bioaerosols, particularly in damp indoor environments, has been associated with allergic sensitization, asthma as well as other adverse respiratory health outcomes. To date, there is limited data on the pulmonary immune mechanisms during subchronic respiratory exposures to fungal bioaerosols. In this study, an acoustical generation system was developed to deliver dry fungal spores to BALB/cJ (a Th2 skewed strain) and B6C3F1/N (a mixed Th1/Th2 strain) housed in a nose-only exposure chamber. The mice were exposed to 10^5 *Aspergillus fumigatus* viable spores or air (control) twice a week for 13 weeks. Cumulative weight gain was observed in both strains and no statistically significant differences were observed between the control and fungal exposed groups within the same mouse strain. Histopathology showed comparable multifocal and pleocellular inflammation, goblet cell metaplasia, arterial remodeling, and mycotic vasculitis in both strains. Flow cytometry analysis of bronchoalveolar lavage (BAL) showed that both mouse strains develop a mixed response helper cell response. $CD4^+$ T cells that expressed the allergy-associated cytokine, IL-13, dominated the responses in both strains. Th1 responses involved in the clearance of inhaled fungal spores were similar in both strains. Interestingly, eosinophils constituted a higher proportion of leukocytes in BAL from B6C3F1/N mice (36%) compared to BALB/cJ mice (9%). These results additionally demonstrate that spore viability, in particular *in vivo* spore germination is an important variable associated with the development of allergic outcomes. Collectively, our findings suggests that subchronic exposure to viable fungal bioaerosols may result in allergic airway inflammation in either mouse strain with comparable immunopathological changes.

ELUCIDATING THE MECHANISMS OF AUGMENTED ALLERGIC RESPONSES WITH DERMAL TRICLOSAN EXPOSURE

NB Marshall, E Lukomska, CM Long, SE Anderson

Allergy and Clinical Immunology Branch, National Institute for Occupational Safety & Health, Morgantown, WV

Triclosan is an antimicrobial chemical incorporated into many personal, medical and household products. 75% of the U.S. population has detectable levels of triclosan in their urine and although it is not typically considered a contact sensitizer, recent studies have begun to link exposure to triclosan with augmented allergic diseases such as asthma and allergies. We examined the effects of dermal triclosan exposure on the skin and lymph nodes of mice and in a human skin model to help identify mechanisms for augmenting allergic responses. Triclosan (0-3% w/v) was applied topically at 24 hour intervals to the ear pinna of ovalbumin-sensitized BALB/c mice. Skin and draining lymph nodes were evaluated for cellular responses and cytokine expression over time. Exposure to triclosan increased the expression of TSLP, IL-1β and TNF-α in the skin with concomitant decreases in IL-25, IL-33 and IL-1α. Similar changes in *TSLP*, *IL1B* and *IL33* expression occurred in human skin. Topical application of triclosan also increased draining lymph node cellularity consisting of activated $CD86^+$ $GL\text{-}7^+$ B cells, $CD80^+$ $CD86^+$ dendritic cells, $GATA\text{-}3^+$ $OX\text{-}40^+$ $IL\text{-}4^+$ $IL\text{-}13^+$ Th2 cells and $IL\text{-}17A^+$ CD4 T cells. Ovalbumin-specific CD4 T cells were also further skewed towards a Th2 phenotype in triclosan-exposed mice. In-vivo antibody blockade of TSLP reduced skin irritation, IL-1β expression, lymph node cellularity, and Th2 responses augmented by triclosan. To our knowledge this is the first report that triclosan induces TSLP expression in skin tissue as a mechanism to augment allergic Th2 responses.

EFFECT OF VALENCE STATE ON CeO_2 NANOPARTICLE TOXICITY IN RATS

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Cerium (Ce), a member of the lanthanide series, is becoming a popular metal oxide for use in mechanical engineering. When in the form of cerium oxide (CeO_2), Ce can exist in both a 3+ and 4+ valence state. This makes the particle an ideal catalyst as a result of improved oxygen storage and releasing capacity. An increase in the number of consumer products containing CeO_2 nanoparticles is expected; thus, understanding the potential toxicity of inhaled CeO_2 nanoparticles during manufacturing is crucial. Previous *in vitro* and *in vivo* evidence has demonstrated that CeO_2 has either antioxidant or oxidant-like properties which is postulated to be due to the nanoparticles ability to transition between 3+ and 4+ valence states. Therefore, we chose to chemically modify the nanoparticles through doping, a process by which an impurity is added to alter the particle's electronic properties, in order to shift the valence state toward 3+. Pure CeO_2 and two doped nanoparticles, 10 mol% gadolinium (Gd) and 20 mol% Gd, were used for this study. Preliminary characteristics indicated that doping results in minimal size and zeta potential changes; however, XPS data implicates that doping dramatically alters the valence state of the nanoparticles. Following characterization of the nanoparticles, male, Sprague-Dawley rats were exposed to 0.5 and 1.0 mg/kg nanoparticles via intratracheal instillation. Animals were sacrificed, bronchoalveolar lavage fluid was collected and lung, liver, and kidney sections were prepared, to determine the effect of valence state on toxicity 1, 7, or 84 days post-exposure. Preliminary results indicate that damage, as measured by elevations in lactate dehydrogenase, occurred within 1 day post-exposure and was sustained 84 days post-exposure; however, no differences were measured between the compounds. Further, there were no differences in inflammatory signaling or lipid peroxidation damage between exposure times or nanoparticle types. Thus, our results implicate that valence state has a minimal effect on CeO_2 nanoparticle toxicity *in vivo*.

STRUCTURE-TOXICITY CORRELATIONS FOR COMPLEX ENGINEERED Ni/SiO_2 NANOMATERIALS USING HIGH-THROUGHPUT ZEBRAFISH ASSAYS

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Functional nanomaterials are developing at an ever accelerating pace and are finding application in a wide range of industrial and consumer products despite evidence that nanomaterials can show elevated toxicity. Furthermore, while bare nanoparticles (NPs) are rarely used in industrial application due to sintering and deactivation effects, embedded NPs (so-called complex engineered nanomaterials, CEN) have found little attention in nanotoxicological studies to-date. This motivates a need for the development of rapid, yet sensitive, high throughput *in vivo* nanotoxicity screening assays.

We are investigating three differently structured Ni/SiO_2 nanomaterials as model CEN with the aim to evaluate different nanotoxicity assays and develop structure-toxicity correlations for CEN that will allow derivation of predictive toxicity models. The CEN are comprised of Ni NPs embedded in or on (non-toxic) porous silica NPs, based on the hypothesis that embedding nickel nanoparticles in silica could reduce or entirely mitigate nanotoxicity while still providing accessibility and hence maintaining functionality of the embedded NP. The three CEN structures are nickel NPs (a) embedded in hollow porous silica shells (hNi@SiO_2), (b) encapsulated in non-hollow, porous silica NPs (nhNi@SiO_2), and (c) deposited on the silica nanoparticle's external surface (Ni-SiO_2). All CENs were thoroughly characterized via TEM, XRD, BET, as well as for dissolution, aggregation, and settling properties, followed by multiple 5-day zebrafish developmental toxicity assays including survival, malformation, hatching, and motility. Zebrafish (*Danio rerio*) were used as a well-suited toxicity model due to prolific breeding and rapid development time. The toxicity results were then correlated with the CEN properties to determine structure-toxicity correlations.

All three nanomaterials mitigated toxicity compared to the respective Ni^{2+} dose and showed high zebrafish survival. Zebrafish motility, which probes persistent neurotoxicity, emerged as a more sensitive assays and revealed lowest toxicity for nhNi@SiO_2, explained by lower nickel dissolution compared to the other two nanomaterials. In contrast, this material showed the highest toxicity of the three CENs in hatching assays, which can be traced back to resistance to aggregation which facilitates uptake through the chorion. Overall, the toxicity results are consistent with a 'Trojan horse' mechanism and point towards complexity and pitfalls when establishing structure-toxicity correlations and the necessity of combining multiple assays to fully assess nanomaterials toxicity.

FIBROGENIC POTENTIAL OF NANO-SCALED CERIUM OXIDE IS AFFECTED BY PHYSICOCHEMICAL PROPERTY CHANGES

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Nano-scaled cerium oxide ($nCeO_2$) is used in a variety of applications, including use as a fuel additive, catalyst, and polishing agent, yet potential adverse health effects associated with $nCeO_2$ exposure remain incompletely understood. *In vivo* studies have shown in a rat model that inhaled $nCeO_2$ deposited deep in the lung and induced fibrosis; however, little is known about how the physicochemical properties of $nCeO_2$, such as size or surface chemistry (e.g. amorphous silica coating), may affect the bio-activity of these particles. Thus, we hypothesized that the physicochemical properties of $nCeO_2$ influence its fibrogenicity. Plasma samples collected from rats at 28 days after intratracheal instillation of 3.5 mg/kg $nCeO_2$ showed increased levels of the fibrotic mediator TGFβ-1, which is released from macrophage and platelets upon stimulation. This effect that was not observed in response to amorphous silica-coated $nCeO_2$ ($amsCeO_2$). Interestingly, platelets isolated from $nCeO_2$-treated rats released significantly more TGFβ-1 than those isolated from control animals, suggesting that platelets may be contributing to $nCeO_2$-induced fibrosis. This was found to be an indirect effect since treatment of control platelets *ex vivo* did not stimulate release of TGFβ-1, regardless of the size or coating of $nCeO_2$. Primary alveolar macrophages demonstrated increased cell death when treated with $nCeO_2$, but not $amsCeO_2$, at doses consistent with those used *in vivo*, while neither particle directly induced TGFβ-1 release from these cells. However, $nCeO_2$ directly induced significant production of collagen I and increased cell proliferation, hallmarks of fibrogenesis, in primary lung fibroblasts *in vitro*, while $amsCeO_2$ failed to do so to the same extent. Furthermore, treatment of the fibroblasts with $nCeO_2$, but not $amsCeO_2$, induced the formation of fibroblastic nodules, which serve as a well characterized *in vitro* model of fibrogenicity. Collectively, these results indicate that differences in the physicochemical properties of $nCeO_2$ may affect the fibrogenicity of this compound, and highlight the utility of “safe-by-design” strategies for preparing engineered nanomaterials.

THE DETERMINATION OF ANTIOXIDANT AND GENETIC RESISTIVITY TO CELLULOSIC COPPER NANOPARTICLES IN *SACCHAROMYCES CEREVISIAE*

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Copper has been used for its antimicrobial properties in medical equipment and agriculture for many years, but by altering the form to copper nanoparticles, the antimicrobial properties greatly increase by displaying greater toxicity. However, with increased use of copper as an antimicrobial chemical, genetic diversity will favor the individuals that are copper resistant. Our results demonstrate that copper resistance in the model organism *Saccharomyces cerevisiae* varies between stain, and that these genetically diverse strains respond to antioxidants differently. When looking at the differences between soluble copper and cellulosic copper nanoparticle (c-CuNPs) resistance, there appears to be different methods of toxicity. Antioxidant treatments with glutathione (GSH) and N-acetylcysteine (NAC) display different levels of resistance depending on the copper source, where NAC is unable to rescue cells treated with c-CuNPs. With respect to internal copper levels, sensitive strains displayed greater concentrations, and cells treated with both copper and an antioxidant displayed ten times greater levels than those treated with copper alone.

POSTER ABSTRACTS

DIFFERENTIAL EFFECT OF NANO-TITANIUM DIOXIDE EXPOSURE ON VASCULAR REACTIVITY

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Engineered nanomaterials (ENM) are anthropogenic materials with at least one dimension less than 100 nm and distinct physicochemical properties and applications. The potential hazardous effects associated with the increased exposure to ENMs remain unclear. Titanium dioxide nanoparticles (nano-TiO_2) are amongst the most widely used ENMs, with applications in clinical, industrial and domestic settings. We have previously reported altered microvascular reactivity following ENM exposure. However, a disparity exists in the literature in terms of ENM toxicity across different levels of the cardiovascular system. The aim of this study was to perform a thorough investigation of ENM toxicity across discrete divisions of the vasculature. We hypothesize that acute exposure to nano-TiO_2 differentially affects the vasculature, with greater impairment of reactivity down the vascular tree. Wire myography was used to assess active tension generation in 2-3 mm rings from the thoracic aorta and the femoral artery. Denudation of the aortic rings was additionally performed to determine the contribution of the endothelium following exposure. Two routes of ENM exposure were selected: coincubation (30 minutes) with increasing concentrations of nano-TiO_2 (10, 100 and 200 μg per 250 μl of medium) and intratracheal instillation (IT; 24 hours prior; 12, 120, or 240 μg nano-TiO_2 suspended in 300 μl of vehicle [saline and 5% FBS]). Rings were preconstricted with phenylephrine (PE; $1x10^{-3}$) prior to challenges with acetylcholine (ACh) ($1x10^{-9}$-$1x10^{-4}$ M), an endothelium-dependent agonist, and sodium nitroprusside (SNP) ($1x10^{-9}$-$1x10^{-4}$ M), an endothelium-independent agonist. In the aorta, instillation of 240 μg of nano-TiO_2 had no effect on vascular smooth muscle response to PE, while responses to ACh and SNP decreased by 41±7% and 28±4% respectively. Coincubation (200 μg) decreased responsiveness of aorta to ACh by 35±4%, while 100 μg and 20 μg of nano-TiO_2 also resulted in a decreased ACh reactivity. Responsiveness to PE increased by 40±5% whereas relaxation via SNP was unchanged. Denudation of aorta resulted in similar responses to all agonists by both unexposed control and exposed aortic rings, further implicating an endothelium-dependent mechanism. In the case of the femoral artery, coincubation with 200 μg nano-TiO_2 abrogated vascular responsiveness to ACh, but PE and SNP responses were unaffected. Femoral artery ACh – induced relaxation showed a greater impairment (40±5%) compared to the aorta following coincubation with nano-TiO_2. These studies provide further evidence of endothelium dysfunction after nano-TiO_2 exposure, increasing in severity moving toward the resistance vasculature.

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THE EFFECTS OF SILVER NANOPARTICLES ON CRAYFISH SYNAPTIC TRANSMISSION

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Marshall University is located next to the Ohio River, which was ranked as the number one river for receiving chemicals in 2013. Because of this, much interest in investigating potential toxicants has been generated. Crayfish are a bioindicator species whose health is indicative of the health of their given ecosystem. The lateral giant neuron in the crayfish is a command neuron that controls a reflexive tail-flip escape response; the context and experience-dependent tuning of this response is critical for survival. Serotonin is known to modulate synaptic responses in this neuron, and social encounters, such as fighting, changes the modulatory role of serotonin. I investigated the serotonergic modulatory changes in this synapse after silver nanoparticle (AgNP) exposure to shed light on potential neurotoxic effects of widely used, but poorly understood nanomaterials. Crayfish were exposed to 1, 5, 10, and 50ppm AgNP for acute (overnight) and chronic (two-weeks) periods. Our data show that stress induced by exposure to AgNP changes excitability of this neuron. Additionally, variability within groups increased as a result of AgNP exposure to higher concentrations, indicating a loss of fine-tune control over this normally organized response system. Integrating these data concerning environmental stress with prior knowledge of behavioral and electrophysiological correlates of social stress we can ask if changes induced in synaptic responses and modulation induced by stressors of different kinds are generalizable.

ARSENIC IMPAIRS MUSCLE STEM CELL FUNCTION AND REGENERATIVE CAPACITY THROUGH NF-KB-MEDIATED MYOFIBROBLAST DYSFUNCTION AND PATHOGENIC EXTRACELLULAR MATRIX REMODELING.

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Arsenic (As(III)) is a global health hazard that contaminates the drinking water of over 140 million individuals worldwide. Epidemiological studies reveal prominent muscle dysfunction and mobility declines following As(III) exposure; yet, mechanisms underlying such declines are unknown. We tested the hypothesis that As(III) drives a maladaptive fibroblast phenotype to promote pathogenic myomatrix remodeling. This remodeling compromises muscle strength and resistance to fatigue, as well as the muscle stem cell (MuSC) niche that is critical to regenerative capacity. Mice were exposed to 0 or 100 µg/L As(III) in drinking water for five weeks before receiving cardiotoxin injections to injure the tibialis anterior (TA) muscle. After 10 days of recovery, the structure and orientation of the TA fibers and ECM of exposed mice was disrupted and functional capacity was compromised after 4 weeks of recovery relative to controls. Decellularized ECM constructs derived from As(III)-exposed mice directed seeded naïve human MuSCs towards fibrogenesis compared to cells seeded onto control constructs. Muscle fibroblasts isolated from uninjured or injured, As(III)-exposed mice had sustained NF-κB promoted ECM remodeling gene expression and expression of fibrogenic thrombospondin-2, and this induction could be observed in naïve fibroblasts exposed to As(III) *ex vivo*. Inhibiting NF-κB during As(III) exposure preserved normal recovery of structure and function following injury, suggesting that NF-κB signaling serves as a mechanism for the deleterious effects of As(III) on muscle repair. In addition, the data suggest that As(III)-induced myomatrix alterations impair MuSC terminal differentiation. These findings may facilitate development of strategies for preventing or reversing muscle declines in As(III)-exposed individuals. *Supported by NIEHS grant R01ES023696.*

PHYSIOLOGICAL EFFECTS OF MCHM ON CONSERVED BIOCHEMICAL PATHWAYS

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Yeast have tremendous advantages to quickly assess modes of action to environmental stressors, such as the industrial chemical 4-methylcyclohexanemethanol (MCHM). Initial treatment of genetically diverse strains of the model organism *Saccharomyces cerevisiae* with MCHM revealed differential sensitivity for toxic exposures between strains. Comparative genetic analysis led to the Med15 protein, a component of the mediator complex that is known to physically interact between stressed regulated transcription factors and RNA polymerase II, as a likely candidate to regulate the cellular response to MCHM. There are three areas of inquiry are key to understanding MCHM biologically. The *med15* knockout strain was extremely sensitive to MCHM. Furthermore, allele swapping of *MED15* from two differentially sensitive strains also showed variable sensitivity in the same genetic background. These alleles have a number of polymorphisms to be explored in the future. Secondly, RNA-seq analysis of the transcriptome from MCHM treated cells shows that the inositol metabolism pathway was distinctly and differentially expressed. The *opi1* mutant strain of the inositol pathway repressor was resistant to MCHM when grown in inositol free media, and inositol at least partially rescues growth of some strains treated by MCHM. In addition there is a synergic growth inhibition when MCHM treated cells are also grown in the presence of drugs that lower inositol such as the mood regulating drugs, lithium and valproate. Structural similarities between MCHM and inositol suggest that cells use the inositol pathway to metabolize MCHM. Reactive oxygen species seem to play a role in the response to MCHM. Mutants for a number of antioxidant pathway genes are sensitive to MCHM, and the antioxidant glutathione and its precursors rescue growth. Bringing these lines of investigation together suggest that alterations of cellular pathway occurs on conserved targets.

REGULATION OF CYCLIN D1 BY ARSENIC AND miRNA INHIBITS ADIPOGENESIS

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Low-dose chronic exposure to trivalent arsenic (AsIII) in drinking water represents a global public health concern with established links to metabolic and cardiovascular disease, as well as cancer. While the link between arsenic and disease is strongly established, further understanding of the molecular mechanisms of its pathogenicity is required. Previous reports have demonstrated the ability of arsenic to interfere with adipogenesis, which may mediate its effects on metabolic disease pathogenesis. We hypothesized that microRNA are important regulators of most if not all mesenchymal stem cell processes which are dysregulated by arsenic exposure to impair lipogenesis. Arsenic increased the expression of miR-29b in white and brown adipose tissue, as well as isolated human mesenchymal stem cells (hMSCs). Exposing hMSCs to arsenic (0.1-1.0 μM) increased miR-29b expression, decreased PTEN a known target repressed by miR-29b and increased expression of cyclin D1 creating a profile promoting proliferation over differentiation. Paradoxically, inhibition of miR-29b increased Cyclin D1 protein levels and forced expression decreased them, both conditions inhibited differentiation. Temporal profiling of Cyclin D1 expression during differentiation suggests that stable inhibition of miR-29b disrupts Cyclin D1 expression changes. Additionally, arsenic was able to repress expression of miR-15a in proliferating and differentiating hMSCs. Overexpression of miR-15a abrogated the effect of arsenic on Cyclin D1. Temporal regulation of Cyclin D1 is critical for adipogenic differentiation and we show here that both arsenic and miRNAs 15 and 29b regulate Cyclin D1 during differentiation, and that alteration of the expression of either miRNA inhibits proper adipogenesis. Because of the tight temporal regulation of Cyclin D1 during differentiation, we suggest a paradigm where either increases or decreases of miR-29b and 15a driven by arsenic can inhibit differentiation.
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THE EFFECT OF POLYMER COATINGS ON THE TOXICITY OF MULTI-WALLED CARBON NANOTUBES

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The unique characteristics of multi-walled carbon nanotubes (MWCNT) that make them ideal for many downstream applications can present adverse health effects. In an attempt to reduce toxicity, decrease human exposure (dustiness), and increase dispersion, companies are applying polymer coatings to as-produced MWCNT (AP-MW). Since comprehensive toxicological data is sparse, the aim for this study was to understand the effect of polymer coating on the pulmonary and systemic toxicity of MWCNT. AP-MW and polymer coated MWCNT (PC-MW) samples from two separate companies were characterized (e.g., length, width, zeta potential, hydrodynamic diameter, etc.). Male C57BL/6J (8 wks) were dosed by pharyngeal aspiration with vehicle (dispersion media), AP-MW or PC-MW at 4 μg (workplace relevant) or 40 μg (induce pathology) and sacrificed at 4 h, 1, 7, 28 and 84 d post-exposure. The characteristics of these materials mimicked those observed from personal breathing zone collections from workers as determined by transmission electron microscopy. The AP-MW from both companies induced pulmonary toxicity. Overall, responses peaked at day 1 and resolved by day 28. The polymer coating from Company 1 had no effect on induced pulmonary cytotoxicity, inflammatory cell influx, or inflammatory gene expression as compared to the respective AP-MW. Interestingly, inflammatory gene expression (*Il6, Cxcl2, Ccl2* and *Spp1*) from pulmonary tissue of PC-MW from Company 2 was attenuated in comparison to the AP-MW. Hepatic indicators of systemic inflammation 24 post-exposure (*Mt1, Mt2, Sap, Saa1, Hp*) were also attenuated by the coating. Histopathological evaluation of PC-MW with respect to AP-MW is ongoing. In neither instance did the polymer coating increase the toxicity of MWCNT and, in fact, the PC-MW from Company 2 decreased the pulmonary inflammatory response. This information is valuable to manufacturers who aim to reduce worker exposure and toxicity.

LOW-DOSE ARSENITE EXPOSURE PROMOTES MITOCHONDRIAL EGFR –MEDIATED DYSFUNCTIONAL ACTIVATION OF DIFFERENTIATING C2C12 MYOBLASTS.

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Chronic environmental exposure to arsenic is a major worldwide public health concern that promotes a number of diseases and morbidities associated with dysfunctional muscle metabolism. Mitochondrial function, long recognized as a target of arsenic toxicity, is essential for muscle maintenance and regeneration following injuries. We recently demonstrated that low-dose arsenite restructures mitochondrial morphology in both skeletal muscle and muscle progenitor cells in vivo with accumulation of dysfunctional elongated mitochondria and a decline of muscle function. Despite the importance of mitochondrial architecture in differentiation, its governance of myogenic cell fate and the molecular mechanisms involved remain poorly understood. Here, we show in C2C12 myoblasts that low-dose arsenite stimulation of dynamin-related GTPase optic atrophy 1 (Opa1) proteolytic cleavage promotes inner mitochondrial membrane topology remodeling and results in inadequate myogenic differentiation. Changes in mitochondrial topology, polarization and cardiolipin (CL) content are concomitant with the mitochondrial membrane accumulation the epidermal growth factor receptor (EGFR), which is known to be activated by arsenite in a number of pathogenic processes. Inhibiting EGFR activity prevented arsenite-stimulated mitochondrial morphology changes, as well as changes in polarization, CL content, and reactive oxygen species generation. Thus, we have identified an important segment of the molecular mechanism that coordinates external environmental cues with mitochondrial intrinsic mechanism that provides the necessary energy and metabolites for differentiation. This mechanism may explain the pathogenic shift in muscle metabolism caused by arsenic exposure. *Supported by NIEHS grant R01ES023696.*

TARGETING SURVIVIN: A SPION-BASED APPROACH TO CANCER DIAGNOSTICS AND THERAPY

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Survivin, an anti-apoptotic protein over-expressed in many types of cancer but undetectable in most healthy tissues, can be regulated *in vitro* with antisense oligonucleotides (ASO). *In vivo,* however, naked ASO are quickly cleared from the bloodstream, rendering them ineffective in clinical settings. ASO can be conjugated to delivery platforms, including superparamagnetic iron oxide nanoparticles (SPION). SPION-based agents have received FDA approval for use as both iron replacement therapy in patients with kidney disease and MRI contrast agents. We hypothesize that coupling ASO targeting survivin to SPIONs will result in a multi-functional, biocompatible theranostic agent that will accumulate in tumors and act primarily on survivin-expressing cancer cells. Seven nanometer SPIONs were synthesized, coated with octylamine-modified poly(acrylic acid), and conjugated with either a survivin ASO (SASO-SPION) or a non-targeting control sequence (NTC-SPION). A549 (human lung adenocarcinoma) cells, which over-express survivin, were used for *in vitro* studies. Fluorescently tagged SASO-SPIONs were internalized by A549 cells following a 24 hour incubation; SPIONs localized to endosomes in the perinuclear regions. The metabolic viability of A549 cells incubated with SASO-SPIONs for 48 hours was significantly decreased, in a dose-dependent manner, compared to both untreated cells and those incubated with NTC-SPIONs. We have demonstrated the internalization of our SASO-SPION platform *in vitro*. Further studies are needed to determine the biodistribution of this formulation *in vivo*. SASO-SPIONs result in a dose-dependent decrease in cell viability a survivin-expressing cancer cell line. As no change in viability was noted at any dose of NTC-SPIONs, this indicates that our SPION platform is not inherently toxic. Further studies are needed on cell lines with varying survivin levels to determine the therapeutic specificity of the SASO-SPION. In addition, the decrease in cell viability should be correlated with a change in survivin protein levels.

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THE HEALTH SCIENCES CENTER RODENT BEHAVIOR CORE: A FACILITY FOR EVALUATING FUNCTIONAL OUTCOMES AT WVU

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Neurological disorders are highly prevalent and are associated with a high medical, financial, and human costs. For example, an estimated 15.5 million American adults are afflicted by stroke, traumatic brain injury, Alzheimer's disease, amyotrophic lateral sclerosis, brain tumor, dementias, epilepsy, Huntington's disease, multiple sclerosis, or Parkinson's disease. Neurological disorders account for an estimated 6.2% of health care expenditures. Further, 2015 projections estimate that nearly 95 million years of healthy life will be lost due to these various neurological conditions. Given that the average lifespan is increasing, this issue is compounded by the increasing size of the at-risk adult population.

To address this pressing medical need, we have developed a Rodent Behavior Core (RBC) for the evaluation of functional outcomes associated with neurological disorders. Rodent models represent an efficient and effective method with which to evaluate disease-related functional alterations and the behavioral impacts of potential therapeutic options. Located in the Health Sciences Center basement vivarium, the RBC is easily accessible to WVU researchers. Apparatus and equipment are available in a suite of dedicated behavior testing rooms for the evaluation of several functional domains including anxiety-related behaviors (elevated plus maze, hole board test, light-dark transition test, marble burying, open field, social investigation), depression-related behaviors (Porsolt forced swim test, sucrose preference, tail suspension), operant conditioning (conditioned place preference chambers, operant chambers), sensorimotor behaviors (adhesive dot removal, cylinder test, paw placement, rotarod, swim test), spatial and non-spatial learning and memory (active and passive avoidance, Morris water maze, radial arm maze), and locomotion (open field, visible platform). We have IACUC-approved Standard Operating Procedures for conducting a variety of rodent behavioral tests. RBC staff are available to consult on experimental design and task selection, conduct and/or train interested researchers in conducting behavioral testing, assist in statistical analyses, and aid in the writing of manuscripts. It is our hope that the RBC will support translational and collaborative research with the end goal of enhancing our understanding of neurological disorder pathology and elucidating possible therapeutic interventions.

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EXAMINATION OF THE EFFECTS OF INHALATION EXPOSURE OF RATS TO WORK SITE FRACKING SAND DUST (FSD) ON ION TRANSPORT IN TRACHEAL EPITHELIUM IN VITRO

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Gas well fracking operations potentially expose workers to fine fracking sand dust during operations. The pulmonary toxicity of inhaled silica dust is well known. The purpose of this investigation was to test the hypothesis that toxicity of fracking sand dust (FSD) could be expressed at the level of ion transport in airway epithelium, which is involved in fluid balance in the lung. Conscious, male Sprague Dawley rats were exposed by inhalation in whole-body chambers to 10 mg/m^3 FSD that had been collected at a gas well site; control animals breathed filtered air. Exposures lasted 6 h/d for 4 d. One day, 7 d and 27 d after the exposure, tracheal segments from euthanized animals were placed in Ussing chambers to measure short circuit current (I_{sc}) and transepithelial potential difference (R_t). Responses to the Na^+ channel blocker, amiloride (apical bath; 10^{-4} M), the Cl^- channel blocker, NPPB (apical bath; 10^{-4} M) and the Na^+,K^+-pump inhibitor, ouabain (basolateral; 10^{-4} M), were then elicited. Exposure to FSD had no effect on basal I_{sc}; R_t also was not affected, indicating that tight junction integrity was unaffected. Amiloride, NPPB and ouabain predictably decreased I_{sc} and R_t. Exposure to FSD did not affect the magnitude of the responses to the ion transport blockers under any of the exposure conditions. The results indicate that, under the conditions of these experiments, inhalation of FSD does not affect I_{sc} or R_t. In conjunction with the results obtained with the ion transport blockers, our findings indicate that ion transport in the airway epithelium of the rat is unaffected by FSD. This suggests that fluid balance in the lung is not perturbed by FSD.

THROMBOSPONDIN-1 IS ELEVATED IN SKELETAL MUSCLE FOLLOWING MWCNT EXPOSURE AND MAY MEDIATE NANOMATERIAL INDUCED LOSS OF ARTERIOLAR REACTIVITY

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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. Nanomaterials, especially multi-walled carbon nanotubes (MWCNT) have been shown to decrease vessel reactivity to acetylcholine, a promoter of endothelium dependent vasodilation. Our objective is to determine the effects of **loss of TSP-1** on microvascular reactivity, and the role of TSP-1 in MWCNT-induced endothelial dysfunction.

Arteriolar responses to ab-luminally applied acetylcholine were assessed in skeletal muscle microvasculature of global TSP-1 null (KO) and wildtype (WT) littermate mice using intravital microscopy and iontophoresis in skeletal muscle following either MWCNT aspiration exposure or sham control. TSP-1 protein levels were measured in mouse skeletal muscle following inhalation exposure to varying concentrations of MWCNT.

Using microiontophoresis, 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, 10-3M. KO animals demonstrated a 23.5% higher maximal dilatory capacity in response to 10^{-2M} adenosine than WT. Following MWCNT aspiration exposure, WT animals achieved only a 5.5% average dilation to the 80nA acetylcholine dose, compared to 31.9% for WT control animals. TSP-1 KO animals demonstrated no significant difference in dilation to any dose of acetylcholine compared to KO controls. TSP-1 protein content was elevated in mouse skeletal muscle following 12hr inhalation exposure MWCNT. These data establish the inhibitory role of TSP-1 for in vivo microvessel vasodilation and suggest that it may be a key component of MWCNT-induced endothelial dysfunction. Understanding the mechanisms behind this process is important as many medical technologies incorporating MWCNT are being developed that interact directly with the cardiovascular system.

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REDUCTION OF *LISTERIA INNOCUA* AND *ESCHERICHIA COLI* ON CONTACT SURFACES BY EXPOSURE TO RADIANT CATALYTIC IONIZATION

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Foodborne illness outbreaks linked to fresh products are becoming more frequent and widespread, which affect 48 million people annually. New sanitizing technologies have emerged in recent years. Radiant Catalytic Ionization (RCI) is an organic form of treatment to disinfect food contact surfaces using reactive oxygen species (ROS). The focus of this study is to analyze the reduction of *L*. *innocua* and *E. coli*, when exposed to RCI. Our results indicate a 90% reduction in the recovery of bacteria with a 60 minute exposure.

LUNG TOXICITY AND ALLERGY RESPONSES IN MICE EXPOSED TO NANOPARTICLE SILVER

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With expansive use of silver nanoparticles (AgNP) in medical applications and consumer products, potential for worker exposure during manufacturing has become a concern. The goal of the current study was to characterize the potential effects of AgNP in an ovalbumin (OVA)-induced allergy model in BALB/c mice. To characterize the effects of AgNP alone, mice were exposed via pharyngeal aspiration (PA) to physiological dispersion medium (DM), 6.1 μg, 18.2 μg, or 73 μg AgNP. Twenty nm diameter AgNP with 0.3% wt polyvinylpovidone coating (NanoAmor, Inc.) were suspended in DM and sonicated before exposures. For all studies lung function was assessed using enhanced pause (Penh); bronchoalveolar lavage (BAL) was performed on the whole lung; BAL cells and fluid were retained for analysis of lung-associated injury, inflammation, phenotyping; and lymph nodes (LN) were harvested for enumeration and immune cell phenotyping. AgNP alone did not result in changes in Penh, while cellular responses in the lung indicated a dose-dependent injury and inflammation by post-exposure day 10, which began to resolve by day 29. Our previous studies have shown that exposure to AgNP prior to OVA-sensitization results in a trend for the development of airway reactivity. In this study, effects of AgNP on the elicitation phase were examined. Animals were sensitized with i.p. injections of OVA (dose) + aluminum hydroxide gel on days 1 and 10. To elicit an OVA-specific response, two PA challenges with OVA were given on days 19 and 28. AgNP were administered by PA on day 27. AgNP did not appear to significantly enhance Penh, and lung-associated LN total cell numbers, BAL cell numbers and IgE levels in serum were not increased above those of the allergy model control (OVA). The results indicate that although AgNP may have a moderate effect on airway resistance in the lung when administered before sensitization, they do not significantly alter the course of allergy development when given either prior to sensitization or during the elicitation phase.

EFFECTS OF PRISTINE AND NITROGEN-DOPED MULTI-WALLED CARBON NANOTUBES (ND-MWCNT) ON REACTIVE OXYGEN SPECIES (ROS) AND CELL CYCLE PROGRESSION

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ND-MWCNT are modified MWCNT with enhanced electrical properties that are used in a variety of applications including fuel cells and sensors; however, the mode of action of toxicity of ND-MWCNT has yet to be elucidated. Recent *in vivo* data showed that ND-MWCNT induced inflammation and fibrosis in mouse lungs to a lesser extent compared to pristine MWCNT. In this study, we compared the interaction of ND-MWCNT or Mitsui 7 MWCNT (MWCNT-7) with human small airway epithelial cells (SAEC) and evaluated their subsequent bioactivities. ND-MWCNT were characterized by transmission electron microscopy, X-ray photon spectroscopy, and Raman spectroscopy, which suggested the presence of defects in the nanotube lattice. The nanotubes were determined to be 93.3% carbon, 3.8% oxygen, and 2.9% nitrogen. A dose-response MTS assay showed that low doses up to 12 μg/mL of ND-MWCNT and MWCNT-7 increased cellular proliferation, while the highest dose of 120 μg/mL significantly decreased proliferation. ND-MWCNT and MWCNT-7 appeared to be engulfed by SAEC at 6h and were fully internalized by 24h. ROS was elevated at 6 and 24h in ND-MWCNT exposed cells, but only at 6h in MWCNT-7 exposed cells. Significant alterations to the cell cycle were observed in SAEC exposed to either 1.2 μg/mL of ND-MWCNT or MWCNT-7 in a time-dependent manner, thus suggesting potential damage or alterations to cell cycle machinery. Our results indicate that exposure to MWCNT-7 or ND-MWCNT induces effects in SAEC possibly through different mechanisms that are potentially related to physicochemical characteristics that may alter their toxicity.

CERIUM DIOXIDE NANOPARTICLES IMPROVE MICROVASCULAR DYSFUNCTION AND OXIDATIVE STRESS WITH HYPERTENSION

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Cerium dioxide nanoparticles (CeO_2) may have anti-oxidant capabilities and are being developed to decrease elevated reactive oxygen species (ROS) levels that are associated with stroke and radiation treatments. However, the current literature suggests that CeO_2 may also be a pro-oxidant. Based on previous observations, we *predict* that in a high ROS *in vivo* environment, CeO_2 will act as an anti-oxidant and may improve microvascular dysfunction by quenching ROS. Spontaneously hypertensive rats (SHR) were studied because they are a well-established model of microvascular dysfunction and local ROS. Wistar-Kyoto rats (WKY) are the appropriate normotensive control for the SHR and were used as the control model for these experiments. Rats were intravenously injected with saline (WKY-Sham, SHR-Sham) or 100 µg of CeO_2 (WKY-CeO_2, SHR-CeO_2) suspended in saline and 5% fetal bovine serum. The reactivity of mesenteric arterioles (4^{th} and 5^{th} order) were studied 24 hr post-exposure via intravital microscopy. Endothelium-dependent dilation was evaluated with acetylcholine (ACh) that was iontophoretically applied to individual arterioles (20, 100 and 150 nAmp). To evaluate the role of ROS, the ACh assessments were repeated in the presence of the superoxide dismutase mimetic, 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPOL, 10^{-4} M) and the hydrogen peroxide scavenger, catalase (CAT, 50 U/mL). Dihydroethidium (10^{-4} M) staining was performed to visualize changes in local arteriolar ROS production. WKY-Sham group responded appropriately to ACh (62±7%, of maximal diameter) and reactivity was unchanged during TEMPOL and CAT incubation (53±8%). The WKY-CeO_2 group responses were also not significantly altered in response to ACh (69±6%), but an augmented response was observed during TEMPOL and CAT incubation (77±6%). Compared to the WKY-Sham group, the SHR-Sham group had a significantly decreased ACh response (32±6%), which was partially restored during incubation with TEMPOL and CAT (51±8%). Following CeO_2 exposure, SHR-CeO_2 endothelium-dependent microvascular dysfunction was significantly improved (48±6%), and reactivity was not further improved during incubation with TEMPOL and CAT (53±8%). Finally, compared to the WKY-Sham group arteriolar oxidative stress was significantly increased in the SHR-Sham group (43±2%). CeO_2 exposure reduced the level of oxidative stress in the SHR-CeO_2 group (18±3%), which was not significantly different from the WKY-Sham group. These results indicate that CeO_2 have potential anti-oxidant activity that improves microvascular dysfunction associated with hypertension.

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HYDROXYL RADICAL GENERATION AND CYTOTOXICITY OF ZINC NANOPARTICLES IN RAW 264.7 CELLS

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Handling nanoparticles presents novel hazards to human health, especially when used commercially before possible toxic effects may be evaluated. Zinc nanoparticles are used in the manufacture of concrete, rubber, and ceramics, and are an additive in food products and paint. Potential toxic effects were studied using RAW 264.7 mouse monocyte macrophage cells. The particles investigated were zinc oxide micron particles (MP), zinc oxide nanoparticles (ZnO NP), zinc metal nanoparticles (Zn NP), and zinc oxide nanowires (NW). The particles' shape, diameter, and percentage of zinc in the sample were characterized. Electron Paramagnetic Resonance (EPR) was used to determine free radical production after H_2O_2 exposure or upon reaction with RAW cells. A CytoTox assay was used to determine LDH levels and a MultiTox assay was used to determine cell viability at 4 h and 24 h exposure times at three different doses: 10, 25, and 50 μg/ml. Comet Assays were performed to determine DNA damage over a 24 h exposure to particles at 10 and 25 μg/ml. EPR results indicated that Zn NP stimulated significantly greater hydroxyl radical (·OH) production than ZnO NP, MP, and NW upon reaction with H_2O_2. After exposure to RAW cells, Zn NP produced significantly greater ·OH radicals than ZnO NP, NW, and MP. LDH levels were increased at 4 h after treatment with Zn NP at 50μg/ml. After 24 h, all particles at 50 μg/ml and ZnO NP and MP at 25 μg/ml caused significant cell damage. Cell viability decreased after 4 h at 50 μg/ml for all particles, and the decreased viability in ZnO NP was significant. After 24 h, all particles at 50 μg/ml and ZnO NP at 25 μg/ml caused cell death. The comet assay revealed that all zinc particles caused increased DNA damage, although it was not significant 24 h post-exposure. Our results demonstrate that Zn NP stimulated greater ·OH production when exposed to H_2O_2 and RAW cells than the other particles. Cellular damage and decreased viability occurred for all particles at the highest dose, and ZnO NP at the medium dose, indicating that ·OH may not be the primary initiator of cell toxicity.

PULMONARY MOUNTAINTOP MINING PARTICULATE MATTER EXPOSURE INDUCES MITOCHONDRIAL MICRORNA DYSREGULATION CONTRIBUTING TO ACUTE CARDIAC DYSFUNCTION

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Heart disease is the leading cause of mortality worldwide and is exacerbated in areas surrounding active mountaintop mining operations. Mountaintop mining generates particulate aerosols and thus creates unique air pollution. Mitochondrial dysfunction has been identified following exposure, yet the causative mechanisms are unidentified. MicroRNAs contribute to homeostatic and adaptive mechanisms and dysregulation contributes to the progression of heart disease. The goal of this study was to determine the effect of mountaintop mining particulate matter (PM_{MTM}) on cardiac function and microRNA dysregulation. Adult male Sprague-Dawley rats and FVB mice were exposed to PM_{MTM} collected from areas surrounding active mountaintop mining operations using intratracheal instillation and pharyngeal aspiration, respectively. Twenty-four hours post-exposure, cardiac functional measurements and mitochondrial isolations were performed. Cardiac dysfunction was indicated in both species compared to their sham control by decreased ejection fraction and fractional shortening. Following mitochondrial isolation, RNA was isolated and RT-qPCR was used to assess microRNA levels within the mitochondria. In both species, miR-378 was increased within the mitochondria following PM_{MTM} exposure. Finally, we identified ATP synthase F0 subunit 6 (ATP6) as a potential target of miR-378 regulation. Immunoblotting identified a decrease in ATP6 protein content in the mitochondria of both species. In conclusion, this study provides evidence that PM_{MTM} exposure increases mitochondrial microRNA content that contributes to mitochondrial dysfunction leading to cardiac dysfunction.

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WITHAFERIN A IS A POTENT INDUCER OF THE NRF2- MEDIATED ENVIRONMENTAL STRESS RESPONSE

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Nrf2 is an inducible transcription factor that guards organisms against toxic stress. This is achieved by inducing cytoprotective enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1*)* to facilitate elimination of toxins. *Withania somnifera (WS)* is a botanical that has been long used in traditional Indian medicine. Though Withaferin A (WA) isolated from *WS* shows therapeutic activity in experimental models, it's potential to induce Nrf2 to protect against toxins is unknown. Wild-type (WT) and Nrf2-disrupted (N0) C57BL/6J mice were gavaged DMSO or 7 mg/kg WA. Human mammary epithelial MCF10A cells, Nrf2 reporter MCF7 cells or WT, N0, Keap1-disrupted (K0) and Nrf2/Keap1-disrupted (K0N0) mouse embryonic fibroblasts (MEF) were treated with graded WA doses. Enzyme expression was quantified by qRTPCR, western blot and luciferase reporter assay. Liver damage provoked by i.p administration of 300 mg/kg acetaminophen was assessed by serum alanine aminotransferase (ALT) and histology. Nrf2 target gene induction was observed in liver, small intestine, lung, colon, brain of WA-treated WT, but not N0 mice. WA administered WT were protected, but not N0 mice, from acetaminophen hepatotoxicity as evidenced by lower serum ALT and liver damage. Nrf2 target gene induction was observed in MCF10A, Nrf2 reporter MCF7, WT and K0 but not in N0 and K0N0 MEF. Transfecting Keap1:Nrf2 (2:1) in K0N0 MEF did not result in induction of NQO1-luciferase activity for WA as it did for sulforaphane and CDDO-Im. Nrf2 nuclear translocation observed in WT MEF post WA. Nrf2 signaling induction by WA was dampened by inhibiting the PI3K pathway. WA (CD= 80 nM) is more potent than sulforaphane (CD=1.5 μM) in inducing NQO1 transcripts in MCF10A. Thus, WA is a potent and novel inducer of Nrf2 that protects cells and organisms against toxic injury in an Nrf2-dependent, Keap1-independent mechanism and may be a useful chemopreventive agent.

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THE EFFECTS OF TRICLOSAN ON CLADOPHORA ALGAE

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The purpose of running this experiment was set up to see if the personal care products that we use in our day to day life had any effect on the plant life and the water quality in Lake Erie. Triclosan is an antibacterial chemical commonly found in personal care products such as soaps, cleaning products and tooth paste. This chemical is toxic to fish crustaceans, embryos and algae. Also when exposed to sunlight it can break down into dixons. Cladophora Algae was chosen because of its ability to go in aquarium and how relatively similar the growing process is in nature with thick, tangled, green patches or coarse hair-like strands attached onto roots, stones and plants. Lake conditions were simulated by making a mesocosm consisting of live fish, algae, snails and more all found from Lake Erie. After culturing the algae that was needed a triclosan stock solution was then created to treat the algae. Labeling the 5 groups. The first group of beakers 1-3 being the control with 0 triclosan solution, the second group of beakers 4-6 with 25ppt stock solution, the third group of beakers 7-9 with 50ppt stock solutions, the fourth group of beakers 10-12 with 75ppt stock solution, and the fifth group of beakers 13-15 with 100ppt stock solution. After a week under these condition a Nalgene glass filter holder with fitted base was used attaching the hand pump to get the purest form of the algae. Continuing this process by running a spect-20 analysis to find the absorbance in the algae. Making sure that the room was completely dark so that the spectrometer didn't pick up any other wave lengths. At the end of the experiment that hypothesis was not supported by the data that was obtained for the 25ppt and the 75ppt but was supported for the 50ppt and the 100ppt. It is important to note that this experiment was performed in a mesocosm which could have influenced the end result. Although this experiment does give a small insight as to what is happening in the lake, further research needs to be done.

CHARACTERIZATION OF AMBIENT AND EXTRACTED FILTER-BASED $PM_{2.5}$ FOR TOXICOLOGY APPLICATIONS

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Toxicology research into ambient fine particulate matter ($PM_{2.5}$) frequently uses filter-based samples that are extracted using protocols that vary between research groups. Limited investigation has gone into the compositional differences that are present between ambient and extracted samples. Here a refined extraction method utilizing elements from previous toxicology studies is outlined with characterization of metals and organics for ambient and corresponding extracted samples.

Ambient $PM_{2.5}$ samples collected throughout Pittsburgh (n=5) were characterized for metal and organic constituents. Co-located samples were extracted through a three step process of removal, concentration, and re-suspension in cell culture media. The $PM_{2.5}$ in media was re-suspended onto filters and analyzed in the same manner as the ambient samples to allow for comparison between ambient and extracted $PM_{2.5}$.

Removal of total mass from the filter was 98.0±1.4%, however a significant loss was observed following complete extraction (concentration and re-suspension). Extraction of metals and organics was lower than total mass and variability was present between specific constituents. Negative correlations were observed between ambient and extracted measurements for total metals and total organics as well as between percent extracted of constituents and total ambient $PM_{2.5}$ mass.

Extraction estimates based solely upon $PM_{2.5}$ mass removed do not reflect the differential removal of constituents during concentration/re-suspension processes and resultant shifts in composition from ambient characteristics. The reduced removal of health relevant constituents with increased ambient mass is of particular concern for toxicology applications. Future regulatory policies that target specific components of $PM_{2.5}$ to mitigate the public health burden must be based on toxicology studies using accurately characterized $PM_{2.5}$.

TOXICOLOGICAL EFFECTS OF INHALED FRACKING SAND DUST ON REACTIVITY AND NEUROGENIC RESPONSES OF ISOLATED RAT TRACHEA

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Hydraulic fracturing (fracking) entails pumping a fluid and sand (silica) mixture through drilled wells to cause the fracturing of shale deep underground, resulting in the release of natural gas. During this process, workers at fracking sites are exposed to large amounts of respirable sand dust as it is prepared for pumping into the well. Inhalation exposure to high levels of crystalline silica is known to produce silicosis. To investigate the toxicological effects of inhaled fracking dust, rats were exposed to fracking sand (collected at a fracking site) at 10 mg/m^3 for 4 days a week and 6 hours a day. Tracheas were isolated from euthanized animals for *in vitro* preparations. In the isolated, perfused trachea at day 7 post-exposure the reactivity to methacholine (MCh) applied to the extraluminal surface of the trachea was increased as compared to air-breathing control animals. On day 7, contractile responses to intraluminally-applied MCh were unaffected by dust inhalation. At 1 day and 27 day post-exposure, reactivity was not altered. In tracheal strips, electrical field stimulation evoked contractions that were not affected by dust inhalation at any of the post-exposure time points. These findings suggest that at 10 mg/m^3, reactivity to MCh and effector nerve function are minimally altered, and that no changes to the integrity of the epithelial cell lining of the trachea have occurred.

GENOTOXICITY OF MITSUI-7, HEAT-TREATED AND NITROGEN-DOPED MULTI-WALLED CARBON NANOTUBES AT OCCUPATIONALLY RELEVANT DOSES

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Multi-walled carbon nanotubes (MWCNT) have many unique applications in medicine, electronics and manufacturing. The low density and small size of MWCNT also makes respiratory exposures to workers likely during the production or use of commercial products. Previous MWCNT exposure data have shown cellular necrosis, cell cycle disruption, chromosome errors and mitotic spindle aberrations in cultured immortalized human airway epithelial cells (BEAS-2B) as well as increased necrosis and colony formation in primary human airway epithelial cells (SAEC) at concentrations anticipated in the workplace. 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 (ND-MWCNT) material has been shown to be less inflammatory *in vivo* than Mitsui-7 native MWCNT (M7-MWCNT). Additionally, exposing M7-MWCNT to extremely high temperatures (HT-MWCNT) removes impurities and reduces their bioreactivity in acellular systems. In order to investigate the potential for lessened *in vitro* toxicity of ND and HT compared to M7-MWCNT, we exposed BEAS-2B and SAEC cells to all three types of MWCNT. All three types of MWCNT showed significant necrosis in BEAS-2B cells after 24-hour exposure to the 24 $\mu g/cm^2$, whereas, after 72 hours of exposure significant necrosis was observed at both the 24 and 2.4 $\mu g/cm$. In the SAEC cells all MWCNT types induced significant necrosis after 24 hours of exposure to 24, 2.4 and 0.024 $\mu g/cm^2$ doses. After 72 hours of exposure in the SAEC cell, all MWCNT types induced significant necrosis at the 24 and 2.4 $\mu g/cm^2$ doses, albeit the ND-MWCNT caused significantly less necrotic death compared to the M7-MWCNT or the HT-MWCNT. The HT and M7-MWCNT induced significant necrosis after 72 hours of exposure in the SAEC cells at the 0.24 $\mu g/cm^2$ dose as well. Cell cycle analysis showed that, in the SAEC cell, a G1/S phase block was induced by all MWCNTs after a 24-hour exposure to 24 $\mu g/cm^2$, but ND-MWCNT had a lessened effect. A G1/S phase block was also observed after a 72-hour exposure to 2.4 $\mu g/cm^2$MWCNT, however; the ND-MWCNT caused significantly less cell cycle disruption than either the M7-MWCNT or the HT-MWCNT. Analysis of the mitotic spindle via confocal fluorescent microscopy revealed that HT, ND and M7-MWCNT induce a significant percentage of aberrant mitoses in the BEAS-2B cells exposed to 2.4 $\mu g/cm^2$ CNT for 24 hours. All carbon nanotubes (CNTs) induced predominantly multipolar mitotic spindles and fragmented centrosomes. Exposure of SAEC cells to 24 $\mu g/cm^2$ inhibited colony formation. By contrast, SAEC cells exposed to 2.4 and .024$\mu g/cm^2$ had an increase in the number and the size of colonies compared to diluent control. Raman confocal analysis determined that ND and HT-MWCNT material was taken up by both cell types. TEM analysis showed HT and M7-MWCNT material within vesicular bodies in exposed BEAS-2B cells. Nuclear penetrations in the BEAS-2B cell were measured via enhanced-darkfield light microscopy and it was found that ND-MWCNT had fewer numbers of individual tubes per 1000 cells compared to the HT-MWCNT in a dose response. This may have ramifications for lessening the toxicity of native MWCNT and protecting workers' health.

COMPARISON OF THE BIOACTIVITY OF CoO AND La_2O_3 METAL OXIDE NANOPARTICLES IN HUMAN SMALL AIRWAY EPITHELIAL CELLS

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Cobalt oxide (CoO) and lanthanum oxide (La_2O_3) nanoparticles are used in a wide variety of industries to improve the quality and efficiency of products, which pose a risk of human exposure. CoO and La_2O_3 have different physicochemical properties leading to differences in their band energy, and it is hypothesized that these changes will result in varying degrees of toxicity. The objective of this study is to understand the bioactivity of both CoO and La_2O_3 nanoparticles using cultured human small airway epithelial cells (SAEC). We investigated cellular toxicity, production of superoxide radicals and alterations in gene expression related to oxidative stress and cellular death following treatment with CoO and La_2O_3 (0.0, 5.0, 25.0, and 50.0 µg/mL) nanoparticles. After characterizing the nanoparticles, we observed using TEM SAEC engulfed CoO and La_2O_3 in increasing amounts as the concentration of nanoparticles increased. CoO was toxic after 6 and 24 h of exposure to 25.0 and 50.0 µg/mL whereas La_2O_3 was toxic after 24 h of exposure to 25.0 and 50.0 µg/mL. CoO produced more superoxide radicals and stimulated total tyrosine and threonine phosphorylation at both 6 and 24 h when compared to La_2O_3 nanoparticles. Taken together, these data provide evidence that the varying degrees of toxicity of CoO and La_2O_3 metal oxide nanoparticles are likely due to their alterations in physicochemical properties such as their band energy.

HUMAN HEALTH AND TOXICOLOGY: DETERMINING OUTREACH NEEDS FOR SCHOOL-AGED STUDENTS

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Some indoor air pollutants are toxic and impact human health. These pollutants include asbestos, biological contaminants, carbon monoxide, tobacco smoke, formaldehyde, lead, pesticides, and radon. Exposure to these toxins can cause medical issues ranging from respiratory infections to brain damage, cancers, and, in some cases of overexposure, death. Awareness through educational programs will allow the exposure reduction of these toxins. In this study, Indiana University of Pennsylvania students were surveyed to assess their knowledge of indoor air pollutants and toxins. This survey data was used to discover areas of misconceptions and to indicate what toxins and academic standards needed further classroom development or educational outreach programs. Ultimately, the results indicated a need for curriculum review according to the Pennsylvania Academic Standards in Kindergarten, Grade 4, and Grade 7. Additionally, the survey research helped to identify that the indoor air toxins radon, carbon monoxide, biological contaminants, asbestos, pesticides, and lead are in need of further outreach programs to help protect school-age students (grades kindergarten to grade 12) from the damaging effects that occur due to exposure.

DOSE- AND TIME-DEPENDENT ASSESSMENT OF HUMAN MESOTHELIAL CELL NEOPLASTIC TRANSFORMATION POTENTIAL FOLLOWING SUB-CHRONIC FUNCTIONALIZED MWCNT EXPOSURE

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Multi-walled carbon nanotubes (MWCNTs) are characterized by asbestos-like fiber morphology, large surface area, and surface chemistries. Exposure results in pulmonary fibrosis, biopersistence, extrapulmonary transport and promotion of adenocarcinoma and sarcomatous mesothelioma. As MWCNTs become widely used, elevated risk of cancer in pleural mesothelium following inhalation exposure is a concern. Long-term exposure risks resulting from surface functionalization of MWCNT on pleural mesothelioma potential is largely unknown, but critically needed. We hypothesized that the effect of surface functionalization of MWCNT on human mesothelial cell neoplastic transformation potential is dependent on dose and duration of exposure. Human immortalized mesothelial cells (MET5A) were continuously exposed to fully characterized prepared (pMWCNT), carboxylated (MW-COOH) and aminated (MW-NH_x) for 6 months at 0.002 and 0.02 μg/cm^2 which are relevant to animal exposure doses. Passage matched saline-only (SAL), dispersant-only (DISP) and crocidolite asbestos (ASB)-exposed cells served as controls. At regular intervals during exposure, each treatment group was assessed for several cancer hallmarks. Results indicated that 1) low dose MW-COOH and MW-NH_x exposure caused significant increases in cell proliferation compared to controls, starting at the third week and persisting over 6 months of exposure. High dose exposure alleviated this effect. 2) pMWCNT, MW-NH_x and ASB cells exhibited significantly greater numbers of soft agar colonies at both doses compared to controls starting at 4 months. Low dose fMWCNT treatment resulted in a more potent colony-forming effect than the high dose, while both doses of asbestos possessed an equipotent effect. 3) Only MW-COOH cells at 6 months exhibited a significant increase in invasion ability compared to all treatments. Lastly, ASB cells displayed the largest transformation frequency while the effect of all the fMWNCT treatment caused moderate effects. In summary, exposure dose, duration and type of surface functionalization determine MWCNT neoplastic transformation potential in pleural mesothelial cells.

EFFECT OF ACUTE AND SUB-CHRONIC INHALATION OF MULTIWALL CARBON NANOTUBES ON PULMONARY FUNCTION

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Multiwall carbon nanotubes (MWCNT) are continually being incorporated into new materials due to their unique physical and electrical properties, yet the full potential risk to workers is not known. While exposure of MWCNT are shown to produce airway inflammation and lung fibrosis in animal studies, the effect of inhalation of MWCNT on pulmonary function has not. In this study we investigated the effects of acute and sub-chronic inhalation of MWCNT on pulmonary function in rats. Male rats were exposed 6 h for 1 day (acute) or 9 days (sub-chronic) to filtered air or 0.5, 1 or 5 mg/m^3 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 lung resistance (R_L) and dynamic compliance (C_{Dyn}) were measured 18 h and 7 d following the end of exposure, and responses to increasing concentrations of aerosolized MCh were obtained. Eighteen h after acute exposure basal R_L was increased and basal C_{Dyn} was decreased; 7 d after exposure basal R_L and C_{Dyn} were not changed. Reactivity to MCh (R_L) was increased and C_{Dyn} responses were decreased at 18 h but not 7 d after exposure. Eighteen h after sub-chronic exposure to basal C_{Dyn} and reactivity to MCh (C_{Dyn}) were increased; 7 d after exposure C_{Dyn} responses were unchanged. The results indicate that acute and sub-chronic inhalation exposure to MWCNT results in changes lung function and airway reactivity, but these changes subside over time.

INHALATION OF MULTI-WALLED CARBON NANOTUBES INDUCES A TRANSIENT INCREASE IN HEART RATE VARIABILITY IN RATS

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Heart rate and cardiovascular function are regulated by the autonomic nervous system, and heart rate variability (HRV) is a marker of autonomic nervous system activity. The prognostic significance of HRV in cardiovascular disease has been widely reported in clinical and epidemiological studies. Recent studies have showed that inhalation of ultrafine particles from ambient air can alter cardiovascular autonomic nerve activity and HRV. To study the effect of pulmonary inhalation of multi-walled carbon nanotubes (MWCNTs) on the autonomic nervous system, male Sprague-Dawley rats were exposed by inhalation to MWCNTs for 5 h at a concentration of 5 mg/m^3. HRV was assessed by analyzing beat-to-beat variations in RR intervals in the time and frequency domains. EKG data were obtained from a telemetry system. Acute exposure to MWCNTs increased the percentage of differences between adjacent R-R intervals over 10 ms (pNN10), the root mean square of the successive differences (RMSSD), low frequency (LF) and high frequency (HF) during the exposure. The ratio of LF-to-HF was not significantly changed. The alterations in HRV were not significant at 1 day- or 7days-post exposure. The results suggested that pulmonary exposure to MWCNTs induced a transient alteration in the activity of the autonomic nerve system.

EXHIBITOR ABSTRACTS

PROTEOMIC ANALYSIS IN THE LUNG AND BRAIN OF RATS FOLLOWING SILVER NANOPARTICLE INHALATION

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Silver nanoparticles (AgNPs) are one of the most widely manufactured nanomaterials for use in industrial, household and diagnostic products, as well as in antimicrobial applications. We have previously shown biodistribution of AgNPs to the brain of rats following pulmonary exposure; however, the potential toxic effects associated with translocation from the lung and the regional distribution within the brain are largely unknown. In this study, male Sprague-Dawley rats were exposed by inhalation to 1 mg/m^3 AgNPs (20 nm diameter, 0.3% PVP coating by wt), or filtered air (control), for 4 hr/d for 14 work days. At 1 and 28 d post-exposure, rats were humanely sacrificed, perfused with saline, and lungs and brains harvested for histology-guided mass spectrometry to determine localization of silver in tissue regions and associated alterations in protein profiles.

Sections (10 µm) of brains and lungs were collected for mass spectrometry and serial sections for H&E staining. Digital microscopy images of the stained sections were annotated. After merging annotated and serial unstained section images, protein and metal ion mass spectra were collected from the annotated areas. Data were analyzed for differences between AgNP treated animals and controls.

Airways and parenchyma were analyzed from the lung at 1 and 28 d post-exposure. Several proteins were found to be differential expressed including peaks at m/z 3266, 3334, 3466, and 3830 with slightly higher levels at day 1 in AgNP exposed animals. Peaks consistent with thymosin β4 and calcyclin were higher in control tissues. A peak at m/z 16916 was absent from day 28 AgNp lung. Minimal changes were observed in brain cortex. In striatum, m/z 4926, 5487, 5636, 6290, 6539, 7649, and 15703 were increased in AgNP exposed animals. In the hippocampus, myelin was found to be greatly increased in AgNP exposed animal while the majority of other proteins were decreased at 28 days. A signal consistent with Ag was detected in the lungs but not the brains of exposed animals using matrix free laser desorption ionization.

PATHOLOGY-DIRECTED MASS SPECTROMETRY DETERMINES PROTEOMIC DIFFERENCES ASSOCIATED WITH CARBON NANOTUBE EXPOSURE IN THE LUNG

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Multi-walled carbon nanotubes (MWCNT) have tremendous industry application however, their unique physicochemical properties may pose health risks after inhalation. For insight into the adverse mechanisms associated with MWCNT deposition in the lung, pathology-directed mass spectrometry profiling was used; a method to identify regionally-specific alterations in proteins, lipids, and metabolites. Mice were exposed by oropharyngeal aspiration to vehicle or MWCNT (10 or 40 ug). Lungs were perfused 24 h post-exposure, inflated with 50% OCT, snap frozen and sections collected on MALDI targets with a serial section stained with H&E. Areas of interest (alveolar and terminal bronchial areas, regions with MWCNT deposition, and regions in treated mice without MWCNT deposition; 50 um in diameter; n=15-20 per section per area) were annotated on H&E stained sections and matched digitally to the corresponding serial section for mass spectra collection to determine proteomic differences. Principal component analyses showed separation of sham from MWCNT-exposed animals and in treated mice in areas with and without deposition. Genetic algorithm classification models indicated spectral classification accuracies of 85% for control, 80% for MWCNT deposition, and 80% for treatment with no deposition. Spectral data (2-40 kDa) indicated numerous peaks separating areas of interest. For example, m/z 10165 and 12973 were increased in areas of MWCNT deposition whereas m/z 3954 was decreased as a result of exposure irrespective of deposition. Segregation was evident in the exposed group as areas without obvious deposition, which either showed no alteration compared to sham (m/z 9450) or the response was blunted compared to areas of obvious deposition (m/z 17097). This methodological approach not only reveals a dynamic range of alterations due to exposure but provides context regionally with respect to MWCNT deposition, thus providing insight into specific mechanisms of MWCNT-induced lung pathology.

A Miniaturized Inhalation Tower to Deliver Small Amounts of API to Conscious Rats.

Aileen House, Walter Horodnik, Jennifer Wilson Wylie, UV Shemesh, Joe Lomask, Ester Carballo-Jane and Peter J. Mauser

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Abstract

Historically, delivering aerosol dry powders to the lungs of pre-clinical species has been challenging. Typically the available techniques require using large amounts of API and/or invasive techniques such as intratracheal administration of the drug. In addition this technique generally requires anesthesia and its incumbent physiological and pharmacological concerns. Besides these less than desirable parameters, the current techniques are not entirely representative of human clinical inhalation and delivery of compound to the lungs. To advance Merck's current capabilities for delivering both liquid and dry powder aerosols to conscious rats, we have developed a state-of-the-art miniaturized method for aerosol delivery that requires only small amounts of API which are delivered to conscious rats. These advances were achieved by integrating the engineering expertise from Buxco Electronics, pulmonary expertise from Merck's Inhalation Platform, and the overall expertise for inhaled drug delivery from Merck's Respiratory Product Development Department. The system that was developed and validated is known as the Buxco Inhalation Exposure Tower or BIET. The BIET consists of a nose-only exposure system attached to an aerosol chamber to minimize skin and fur contamination, eliminates the need for anesthesia, and minimizes compound use. BIET can also be used to measure in real-time changes in respiratory physiology during drug delivery. Measuring these respiratory physiological parameters provides an index that allows the identification of potential liabilities that may be associated with the inhaled novel chemical entities while at the same time providing real time minute ventilation during drug delivery. The ability to measure real time ventilation is an advance over typical inhalation delivery systems. Now the delivered dose (DD) of compound no longer has to be calculated using estimated respiratory minute ventilation (RMV) based on the animals body weight but can be measured in real time. In the present studies, the Allay™ restraint collar[1] was used in conjunction with the nose-only plethysmograph to measure ventilation in rats exposed to a variety of air flows in the range traditionally used in larger safety and toxicology towers. This allowed us to assess the effect of different airflows through the BIET and compare the RMV in the chambered animals compared to animals under normal ventilation conditions measured as a control outside the unit but in plethysmographs. Male Wistar rats (143-213 g) were placed in the Allay™ restraint collar/nose-only plethysmograph for 30 min per experiment while breathing room air outside of the BIET. Tidal volume (Vt, ml), respiratory rate (f, breaths/min) and minute ventilation (RMV, ml/min) measured on a breath-by-breath basis and showed remarkable stability over this 30 min control period. RMV decreased slightly over time as the rat accommodated to the restraint. Average values for Vt (2.10 ± 0.14, ml), f (131 ± 8, breaths/min) and RMV (183 ± 34, ml/min) were within values reported in the literature. When the rats were placed in the BIET, on-line ventilation data was obtained as the rats breathed air in the tower at different flow rates that would be used during exposure of the rats to inhaled drugs. Values for RMV at air flow rates ranging from 2.5-8.0 liters per minute ((LPM) circulated through the tower were reduced when compared to RMV values obtained during normal ventilation outside of the tower (183 ± 34, ml/min in the BIET, 259 ± 41, ml/min outside). These differences were not statistically different. When traditional methods were used to calculate RMV based on animals weight for the animals on study, these calculated RMV values were greatly reduced in comparison to those actually measured. In conclusion, the BIET tower using the Allay™ restraint collar and nose-only plethysmograph offers an advantage over conventional systems by reducing drug requirements; avoid reliance on estimations of RMV for calculating DD and eliminating anesthesia. This miniaturized tower system yields high quality ventilation data for an extended period of time and can be used to assess the respiratory effects of drugs given by nose-only inhalation through the inhalation tower. The ability to maintain normal ventilation over a large range of airflow through the tower enables Merck to be able to use small amounts of API for evaluations using this tower in conscious rats.

1 House, A., R. Shafer, Y. Shemesh, J. Lomask, R. W Chapman, P. J Mauser Use of the ALLAY™ Restraint Collar to Facilitate the Measurement of Ventilation in Conscious Rats. Am. J. Respir. Crit. Care Med. A4049, 2013

Introduction

- Aerosol delivery of dry powders to the lungs of pre-clinical species has been historically challenging. Typically it requires using large amounts of API and invasive techniques such as intratracheal administration under anesthesia. These techniques are not entirely representative of clinical inhalation.
- In order to advance Merck's current capabilities for delivering both liquid and dry powder aerosols to conscious rats, we have developed a state-of-the-art miniaturized method of aerosol delivery using small amounts of API delivered to conscious rats by integrating the expertise from Buxco Electronics, Merck's Inhalation Platform, and Merck's Respiratory Product Development Department.
- The system developed and validated is the Buxco Inhalation Exposure Tower (BIET). The BIET is a nose-only exposure system that minimizes skin and fur contamination, avoids anesthesia, and minimizes compound use. In addition BIET has the ability to measure in real-time changes in respiratory physiology during delivery.

Methods

Animals:-

- Experiments were performed on male Wistar rats ranging in weight from 145-220 g.
- The rats were allowed to acclimate to the restraint for 5 days before measurements were obtained.

Allay™ Restraint Collars:-

➢ **Step 1:**

- The Allay™ restraint collars are color coded to fit different size rats (Fig 1). The appropriate size collar was selected to fit snugly over the rat's neck, just behind the ears and in front of the shoulders.

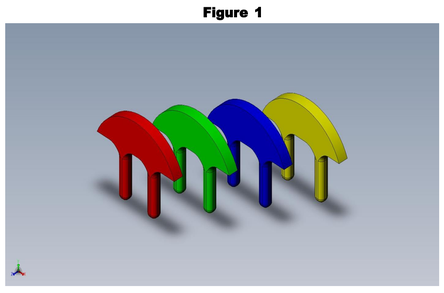
➢ **Step 2:**

- The rat was positioned in the plethysmograph chamber and the Allay™ restraint collar was inserted through the slot at the top of the plethysmograph and positioned over the neck of the rat just behind the ears (Fig 2).

➢ **Step 3:**

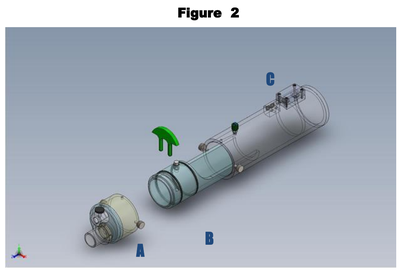
- The nose-cap was attached to the front end of the plethysmograph until the O-rings locked in place. Care was taken to ensure that the nose of the rat passed through the latex nose-seal (Fig 2). The rear of the plethysmograph was then sealed until the O-rings locked in place.

Figure 1



Allay™ restraint collars for use in rats. Yellow is the largest 0.75", blue is intermediate 0.70", green is smaller 0.65"and red is the smallest. 0.060"

Figure 2



Nose-only plethysmograph adapted for use with the Allay™ restraint: (A): nose-cap with latex nose seal, **(B)**:Allay™ restraint collar, **(C):** wire mesh screen

Experiment design:-

- Experiments were performed to evaluate the quality of the ventilation data while exposing the rats to various flow rates in the BIET tower.

➢ **Step 1:**

- Ventilation was measured for 30 min while breathing room air. Rats were then placed in the tower. Fig (3)

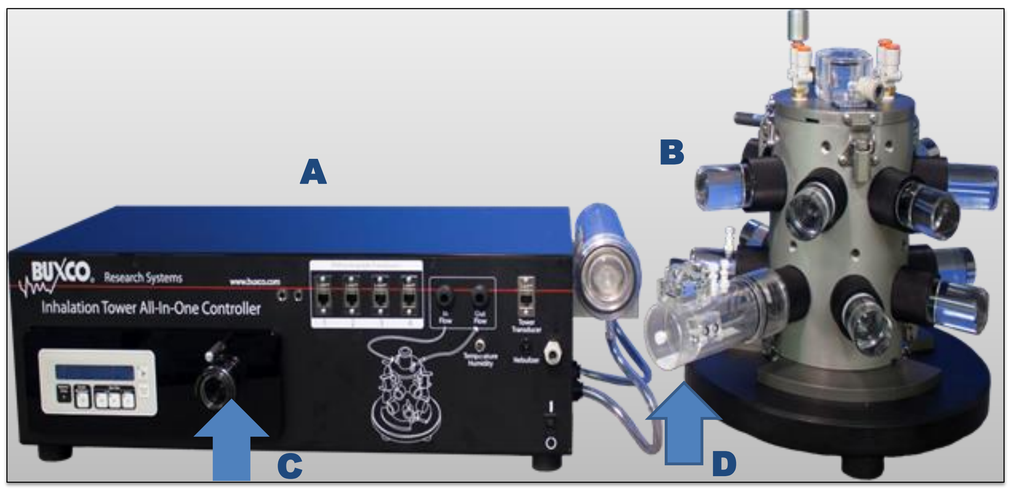
➢ **Step 2:**

- The flow rate was set for a pre-determined flow rate. Ventilation measured over a 20 min period while breathing air from the BIET at 7 flow rates ranging from 2.5 to 8.0 L/min.
- Ventilation in the tower was compared to ventilation breathing room air.

Measurement of Ventilation:-

- Airflow was measured as the pressure drop across a wire mesh screen placed in a hole in the wall of the plethysmograph using a differential pressure transducer (TRD5700) and volume signals were derived by integration of this airflow signal.
- Volume calibrations were performed using the FinePointe Calibrator.
- With the rat in the plethysmograph, tidal volume (Vt, ml), respiratory rate (f, breaths/min) and minute volume (RMV, ml/min) were measured for each breath and the average of this data was displayed every minute.
- All data was generated using Buxco's FinePointe™ System.

Figure 3



All-in One Controller and BIET:
(**A**): All-in-Once Controller Unit (**B**): Tower (**C**): FinePointe Calibrator (**D**) Plethysmograph

Results

Table 1

- **Table 1 shows ventilation at different flows rates while the pressure in the tower is maintained at zero.**
- **These flow rates are approximately 1.5 X and 5.0 X the average RMV of a 200 g rat.**

Flow (LPM)	LPM/port	Vt (ml)		f (breaths/min)		MV (ml/min)		BWT
		Air	Tower	Air	Tower	Air	Tower	(g)
2.5	0.5 *	2.18 ± 0.25	1.87 ± 0.24	126 ± 9	117 ± 10	265 ± 26	212 ± 26	213 ± 5
3.0	0.6	1.68 ± 0.22	1.41 ± 0.15	147 ± 6	107 ± 6	217 ± 77	136 ± 48	148 ± 2
4.0	0.8	2.09 ± 0.21	1.92 ± 0.21	135 ± 5	110 ± 5	249 ± 26	184 ± 25	147 ± 2
5.0	1.0	2.16 ± 0.06	1.62 ± 0.13	125 ± 7	92 ± 4	282 ± 35	149 ± 18	143 ± 3
6.0	1.2	2.06 ± 0.13	1.96 ± 0.22	138 ± 8	117 ± 10	286 ± 34	240 ± 43	145 ± 1
7.0	1.4	1.98 ± 0.07	1.88 ± 0.67	141 ± 12	98 ± 4	272 ± 18	118 ± 3	148 ± 2
8.0	1.6	2.71 ± 0.70	1.87 ± 0.24	104 ± 8	105 ± 3	239 ± 72	240 ± 72	155 ± 3

*** The flow coming from the port is equal to Flow/# of ports being occupied.**

Table 2

- **Table 2 is a comparison of calculated Respiratory Minute Volume (RMV) to measured RMV**
- **RMV = Respiratory Minute Volume (L/min.) = (0.499 (BW)^0.809)[1]**

	Air		Tower	
LPM/port	Calculated RMV	Measured RMV	Calculated RMV	Measured RMV
	(L/min)	(L/min)	(L/min)	(L/min)
0.5	0.129	0.265	0.129	0.212
0.6	0.090	0.217	0.090	0.136
0.8	0.089	0.282	0.089	0.184
1.0	0.087	0.249	0.087	0.149
1.2	0.088	0.286	0.088	0.240
1.4	0.090	0.272	0.090	0.118
1.6	0.094	0.281	0.094	0.204

[1]Bide, R.W., Armour, S.J., and Yee, E., Allometric respiration/body mass data for animals to be used for estimates of inhalation toxicity to young adult humans. J.Appl. Toxicol. 20 (4):273-290, 2000)

Conclusion

- **The calculated RMV for rats restrained and breathing room air or from the tower underestimates the actual measures RVM.**
- **Measured RMV is consistent over the range of flows evaluated.**
- **The flow range at the port (0.5 to1.6LPM) covers a range in flow that is recommended to clear exhaled atmosphere and avoid rebreathing and oxygen depletion.**

Discussion

- **The BIET tower using the Allay™ restraint collar and nose-only plethysmograph offers an advantage over conventional systems that rely heavily on estimations of RMV for calculating DD. This miniaturized tower system yields high quality ventilation data for an extended period of time and can be used to assess the respiratory effects of drugs given by nose-only inhalation through an inhalation tower. The ability to maintain normal ventilation over a large flow range in the tower will enable Merck to be able to evaluate small amounts of inhaled API in conscious rats.**

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