ALLEGHENY-ERIE REGIONAL CHAPTER
of the
SOCIETY OF TOXICOLOGY

ANNUAL REPORT: 2017-2018

June 1, 2017-May 31, 2018

I. Officers:

Office	2017-2018	2018-2019
President:	Todd Stueckle	Aaron Barchowsky
President-Elect:	Aaron Barchowsky	TBD
Vice President:	Aaron Erdely	Stephen Leonard
Secretary:	Robin E. Gandley	Robin E. Gandley
Treasurer:	William J. Mackay	William J. Mackay
Past President	Jim Antonini	Todd Stueckle
Councilors:	Mark Weisberg	Mark Weisberg
	Kristen Russ	Vamsi Kodali
	James P. Fabisiak	James P. Fabisiak
	Tim Nurkiewicz	Tim Nurkiewicz
	William J. Mackay	Alexander Ufelle
	Elaine Freeman	TBD
Postdoctoral Representative:	Vamsi Kodali	Elizabeth Bowdridge
Postdoctoral Representative Elect:		Briana De Miranda
Graduate Student Representatives:	Katie Roach	Katie Roach Shelbie Burchfield
K-12 Outreach:	William J. Mackay	William J. Mackay
RC4 Liaison	Aaron Barchowsky	Aaron Barchowsky

President's comment: *The past year was very successful for A-E SOT. This is directly attributable to the continued energy and commitment of the current leadership. A-E SOT has been successful in providing the region with an annual high impact scientific meeting that highlights toxicology research in academia, industry, and government and in the training and development of young scientists in the field of toxicology. We were successful in growing the membership and providing participation from more regional undergraduate institutions, which has identified several opportunities for ToxScholar and other outreach opportunities in the coming year.*

II. Committees:

Awards:	Todd Stueckle
Communications:	Mark Weisberg
Education:	Aaron Barchowsky
Finance:	William Mackay
Membership:	Tim Nurkiewicz
Nominating:	Jim Antonini
Program:	Aaron Barchowsky, Todd Stueckle
Web:	Katie Roach
RC4 Representative:	Aaron Barchowsky

III. Activities:

a) 2018 A-E SOT Annual Meeting

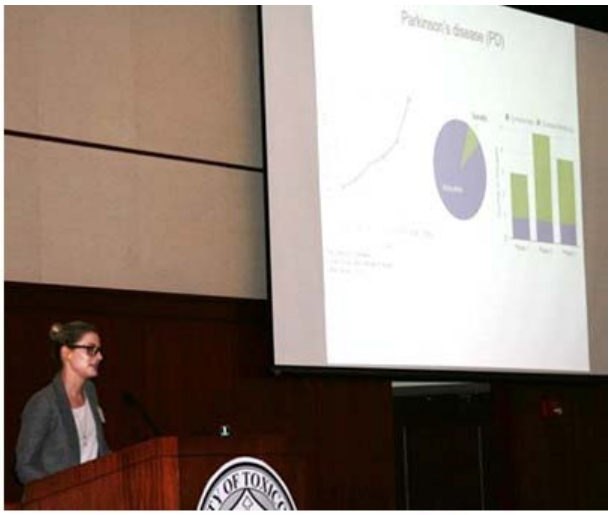
Location / Date: Erickson Alumni Center, Morgantown, West Virginia. May 30-31th, 2017.
Registered Attendees: 98

Highlights:

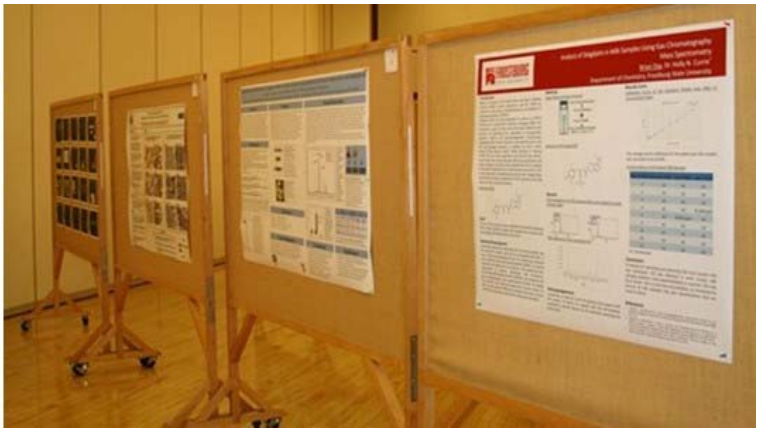
1) The three symposia during the 2-day conference on '*Emerging Issues in Toxicology*' were headlined by the invited speakers: Drs. James Luyendyk, Justin Colacino, and Luis Ortiz who each presented in one of the scientific sessions followed by lively Q&A sessions from attendees.

2) On behalf of the West Virginia University Health Science Center and Director of the WVU Toxicology Working Group, Dr. Tim Nurkiewicz, welcomed all attendees to the meeting, highlighting WVU's continued commitment and enthusiasm for hosting the A-E SOT conference for the past few years, and indicated the ongoing support from WVU for A-E SOT endeavors to help facilitate academic, government, and industry collaborations in the toxicological sciences.

3) In addition to our keynote speakers, the symposia continued our Chapter's focus on education and scientific access. As such, many of our postdoctoral fellow and graduate student trainees were able to present platform presentations in this year's symposia. We were also able to expand and broaden our invitation and hosting abilities for area HS and undergraduate students and their mentors to participate in the conference and our Lunch with an Expert program (please see below). By expanding our reach to Northeast Ohio Medical University and a renewed commitment to recruiting and increasing Pittsburgh area student involvement, we had 45 accepted abstracts, our highest count ever for our Annual Meeting (please see supplemental program).



4) Following our first day and two scientific sessions, we held a poster session over refreshments. Overall, there were more than 32 poster presentations. Please see the supplemental program for the scientific abstracts. Posters and presentations were judged by Drs. Luyendyk and Colacino (invited speakers) and a panel of members of A-E SOT leadership.



5) We set a goal for 2017-2018 to increase participation of undergraduates at the annual meeting as a means to address SOT's strategic objective of increasing undergraduate recruitment and retention in toxicological research. Building off of the enthusiasm of recruited undergraduate mentors who attended the meeting last year, we increased the number of participating undergraduate programs to six (Edinboro University, Frostburg State University, Indiana University of Pennsylvania, Northeast Ohio Medical University, Slippery Rock University, and West Virginia University). Three of the six undergraduate students attending presented posters, along with one high school student. We already are planning two different ToxScholar outreach activities for 2018-2019 as a result of undergraduate mentor attendance at the Annual Spring meeting.

6) The sixth year of "Lunch with an Expert" was again a successful highlight for student attendees. This year's event at the annual meeting was well received with participation from seven graduate students, 4 undergraduates, and 8 mentors. Mentors included postdoctoral fellows who addressed questions from undergraduate progression through admission to graduate school and transition to careers. There was lively discussion between government, academic, and industry mentors and the students regarding the current state of toxicology research and practice, as well as future career opportunities. The feedback both students and mentors participating in the event was enormously positive, and provided a significant professional development opportunity for the next generation of

toxicologists. We will definitely continue providing this valuable activity at the annual meetings and are very grateful for the generous support from SOT to make this luncheon for our trainees possible.

7) <u>2017-18 A-E SOT Awards:</u> Based on our healthy budget and the large increase in attendance, the A-E SOT Executive Committee saw it fit to increase the number of awards and the dollar value given out to a large and diverse pool of student presenters. In addition, we began two new awards, namely **Best Undergraduate Poster** and **Mentor of Best Undergraduate Poster** (inset) to acknowledge a hard working student and their mentor; and to serve as a Spring Meeting recruitment tool for future undergraduate student researchers.

2018 Maryanne Stock Student Research Award
Alaeddin Bashir Abukabda
West Virginia University
Project: *"Maternal Pulmonary Exposure to Titanium Dioxide Nanoparticles Alters Placental Hemodynamics.*

2018 Graduate Student Travel Awards
Meghan Bucher
University of Pittsburgh

Kelly Smith Fraser
West Virginia University/NIOSH

2018 Postdoctoral Fellow Travel Award
Dr. Bharat Bhushan
University of Pittsburgh

2018 Best High School Student
Samuel Zlotnikov
Winchester Thurston School

2018 Best Undergraduate Poster
Caroline Leadmon
West Virginia University
Mentor: Dan Panaccione

2018 Best Graduate Student Poster Presentation
Timur Khaliullin
West Virginia University/NIOSH
Mentor: Dr. Anna Shvedova

2018 Best Graduate Student Oral Presentation
Meghan Bucher
University of Pittsburgh

2018 Best Postdoctoral Fellow Oral Presentation
Dr. Briana De Miranda
University of Pittsburgh

2018 Best Postdoctoral Fellow Poster Presentation
Dr. Bridget Hindman
National Institute for Occupational Safety and Health

2018 Best Young Investigator Poster
Dr. Mohamed Shoeb
National Institute for Occupational Safety and Health

8) This past year A-E SOT was able to acquire funding from SOT to aid in student travel and lodging for our annual meeting attendance. The funding was beneficial and provided financial assistance to several students from area universities to attend 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. We also used Chapter funds to cover the cost of attendance for high school students from Morgantown HS and Winchester Thurston HS in Pittsburgh, PA.

9) Following our poster session on Day 1, the Chapter hosted a small informal reception for all attendees. This was held to further increase interaction between our invited speakers and meeting attendees. Support for this reception came from our generous sponsors.

*** ***For complete 2018 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, we overhauled our website in October 2012, and this is now an ongoing process (or living document). We recently updated the website to include information on the annual meeting, the current award winners, and 2018-2019 officers.

VI. 2019 Annual Meeting: We have tentatively selected the first or second week of May 2019 for next year's meeting. This meeting will be held at the University Club at the University of Pittsburgh. Support for this meeting venue will be solicited from the University administration and our sponsors. Despite several successful meetings at West Virginia University, the return to a Pittsburgh meeting is an effort to increase participation from the Pittsburgh-based SOT members and industry partners. This should also increase our service to the region and continued vitality of A-E SOT.

VII. New Meetings/Activities: For the sixth year, A-E SOT joined with the Michigan and Lake Ontario Regional Chapters for a joint reception at the National SOT meeting in San Antonio. Virtually all A-E SOT members in attendance at the SOT annual meeting attended the joint reception and the event was considered a success by all chapters, even better than last year's three-chapter reception. These joint receptions have fostered productive interactions between the chapters, including invitations to Drs. James Luyendyk and Justin Colacino from the Michigan Chapter to present key note talks at the A-E SOT meeting. We had plenty of food on hand despite the large turnout, mostly attributed to the excellent planning and budgeting a few months ahead of time by members of each Chapter's executive committees.

2018 Alleghany-Erie, Lake Ontario, & Michigan Chapter Joint Reception

VII. Budget/Bank Statement: Generous donations from sponsors (The WVU Department of Physiology & Pharmacology, RJ Lee Group, LLC) and an increase in meeting attendance resulted in ~$22,105 (June financial statement) in the A-E SOT account at the time of this report. We are operating in the black for six consecutive years while simultaneously increasing the size and impact of our annual meeting, and introducing new Chapter activities and public outreach. To bring our finances back to the level of the last few years, 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, young investigators, and undergraduate students 2) continue to develop/increase our annual meeting towards a "Current Concepts in Toxicology" meeting, 3) continue our joint reception with the Michigan and Lake Ontario Regional Chapters at the Annual meeting and 4) increase our outreach impacts primarily targeting undergraduate students and their mentors partially via the ToxScholar Program.

VIII. Outreach: A-E SOT has long-standing support for the Pennsylvania Junior Academy of Science (PJAS) and continued this support with several $100 awards for presenting students (see Attachment). In addition, our Graduate Student representative, Katie Roach, traveled to Trinity Christian School to assist with judging their high school STEM Fair. A-E SOT gladly handed out several $50 awards to deserving students along with vouchers for themselves and a parent to attend the A-E SOT Spring Meeting (see attached letter).

For 2017-2018, William Mackay led our K-12 outreach, despite the loss of funds from National SOT. Several new avenues have been discussed and are being implemented. These ideas include connecting with the past President of the West Virginia statewide science teachers association to develop contacts for outreach in West Virginia and continue to develop plans with both WV and PA Science Bowl competitions to supply A-E SOT members as volunteers and small financial support. In addition, we had a very enterprising high school student who self-funded the projected he presented in a poster at the annual meeting. He efforts and work were so impressive that the Leadership unanimously agreed to create a special High School Student Award for him that was recognized during awards ceremony.

Outreach to undergraduates and their mentors was a priority goal of our Chapter for the 2017-2018 year. This last year Dr. Stueckle identified several faculty at regional undergraduate universities with known activities in toxicology research. Targeted letters were sent out to faculty to introduce them to A-E SOT and invite them to the Spring Meeting. This effort was moderately successful as it attracted several faculty and undergraduate students to the Spring Meeting. This included participation from six undergraduate programs (Edinboro University, Frostburg State University, Indiana University of Pennsylvania, Northeast Ohio Medical University, Slippery Rock University, and West Virginia University). In face-to-face chats at the Meeting, these faculty were very pleased with the overall theme and openness to student involvement. These faculty plan to bring more undergraduates to next year's meeting. Faculty and students from Slippery Rock University are a special example as Officer Aaron Barchowsky used a ToxScholar visit to Slippery Rock University to recruit a faculty member, Alexander Ufelle, as a new Councilor and he brought an undergraduate student, Molly Schreiner, to attend the meeting and present her work. Molly's project is a collaboration between Dr Ufelle and Past-President Todd Stuekle at NIOSH. A-E SOT will pursue target at least two ToxScholar visits to a regional college in West Virginia and one in Western Pennsylvania this coming year as the most effective means of growing the undergraduate programming.

VIV. Other: We achieved some of our goals for increasing the breadth of the Chapter to include more participation from Pittsburgh area industry and academia. This included recruiting President-elect candidates from Pittsburgh area industry and regulatory toxicologist and more graduate students and postdoctoral fellows in 2017-2018. Unfortunately, our President-elect had to leave the Pittsburgh area and we are holding a special election to replace him with other industry leaders. We amended our bylaws to increase the number of graduate student and postdoctoral representatives to both include participation from Pittsburgh and to create a path of succession in the positions. It is likely that we will hold the annual meeting in the Pittsburgh area for the next two years and will be strategically developing the meeting programs to appeal to our industry and regulatory members. We believe this will foster additional networking for research opportunities and applied student internships.

32nd Annual Meeting of the Allegheny-Erie Society of Toxicology Regional Chapter

Erickson Alumni Center
West Virginia University
Morgantown, WV
May 30-31, 2018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Emerging Issues in Toxicology

SYMPOSIA SCHEDULE

DAY ONE: Wednesday, May 30

9:00–9:45 Registration
10:00–10:05 Welcome and Announcements
10:05-10:15 WVU Welcome; Dr. Clay Marsh, Dean, School of Medicine

***SYMPOSIUM 1*:**

10:15–12:30 **Systems Toxicology**

10:15 Introduction/Symposium Overview (Dr. Tim Nurkiewicz)

10:30 **Keynote Speaker: Dr. James Luyendyk; Michigan State University:** Mechanisms Linking the Hemostatic System to Liver Toxicity and Repair

11:30 **Kelly Fraser; West Virginia University**: Comparative Assessment of *In Vivo* Toxicity Induced by Multi-Walled Carbon Nanotubes and Nanofibers from U.S. Facilities

11:50 **Eiman Aboaziza**; **West Virginia Clinical Translational Science Institute:** *In Vivo* Immune-Spin Trapping Detection of Free Radicals in Cardiac and Hepatic Tissues following Acute Exposure to Electronic Cigarette Vapor

12:10 **Elizabeth Bowdridge; West Virginia University**: ENM Inhalation during Gestation Disrupts Plasma Estrogen and Vascular Kisspeptin Reactivity

12:30–1:15 LUNCH

***SYMPOSIUM 2*:**

1:15–3:30 **Stem Cells in Toxicology and Disease**

1:15 Introduction/Symposium Overview (Moderator Dr. Elizabeth Bowdridge)

1:30 **Keynote Speaker: Dr. Justin Colacino; University of Michigan:** Environmental Exposures and Stem Cell Reprogramming: Understanding Racial Disparities in Triple Negative Breast Cancer

2:30 **Teressa Anguiano; University of Pittsburgh:** Arsenic Disrupts Muscle Stem Cell Determination through Fibroblast Mitochondrial Maladaptation that Directs a Dysfunctional Extracellular Matrix Memory

2:50 **Alaeddin Abukabda; West Virginia University:** Maternal Pulmonary Exposure to Titanium Dioxide Nanoparticles Alters Placental Hemodynamics

3:10 **Dushani Palliyaguru; University of Pittsburgh:** Broccoli-derived Sulforaphane Prevents Formation of Mammary Tumors in Rats Exposed to 17β-estradiol

3:30 **Don Ewert, RJ Lee Group Inc:** GHS Hazard Inversion Techniques; Measuring Bioavailability

3:50–4:00 Break
4:00–6:30 Poster Session – Networking – Refreshments
6:30-8:30 Group Dinner: Apothecary Ale House and Café (see enclosed map)

DAY TWO Thursday, May 31

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

***SYMPOSIUM 3*:**

9:30–11:30 **Exosomes and Signals Undetected**

9:30 Introduction/Symposium Overview (Moderator Dr. Kristen Russ)

9:45 **Keynote Speaker: Dr. Luis Ortiz, University of Pittsburgh:** Dissecting the molecular mechanisms by which bone marrow mesenchyma helps environmentally induced lung injury: MSCs Use their ARRMS to throw vesicles

10:45 **Meghan Bucher; University of Pittsburgh:** Viral-Mediated Dysregulation of Vesicular Monoamine Packaging is Toxic to Dopaminergic Neurons

11:05 **Briana De Miranda; University of Pittsburgh:** Environmental Mitochondrial Toxicants and LRRK2; a New Gene-Environment Interaction in Parkinson's Disease

11:25 **Mohammad Shoeb; NIOSH:** Measurement of Telomere Length and Regulatory Genes in Peripheral Blood Monocytes and Lung Tissue as a Biomarker for Response after Inhalation Exposure to Occupational Particles

11:45–12:45 "Lunch with an Expert" & Networking

12:45–1:00 Awards

1:00 Closing Comments and Adjourn

Undergraduate Program and Information

Dear Undergraduate Researchers and Research Mentors,

On behalf of the A-ESOT Leadership Committee, welcome to the 32nd Annual A-ESOT Spring Meeting. We are thrilled to have your interest and involvement within the interdisciplinary science of toxicology! In that spirit, we have numerous activities planned, and we encourage you to take full advantage of them.

Important Information

1) **Registration**: It's free for undergraduate students and mentors! By Friday May 18th please email both Dr. Stueckle and Dr. Barchowsky the **name** and **affiliation** of those students and mentors that plan to attend the meeting. We need a head count for space and food estimates. You may also register on-site if needed. Two lunches, Thursday breakfast, and break refreshments are provided with your registration. If you have already registered, thank you!
2) Please RSVP for Lunch with an Expert (see below) by emailing Katie Roach, our Graduate Student Representative (wvq1@cdc.gov). In your email, indicate what interests in toxicology you have to best pair you up with an Expert.
3) **Travel Reimbursement:** A-ESOT has secured funds from national SOT to solely support your travel expenses (e.g. hotel room, gas, etc). To be reimbursed for your expenses, please submit all original receipts to Dr. Todd Stueckle.
4) **Hotel Information:** If you have not already done so, please book your hotel room. We have a block of rooms set aside under the name 'Society of Toxicology' at the Holiday Inn, 1188 Pineview Drive, Morgantown WV. 304 241-6649.

Undergraduate Program:

1) Hot Topic Scientific Talks from Invited Speakers – We have three renowned keynote speakers lined up to speak on topics including systems biology, stem cells, and exosome responses in toxicology. In addition, we expect to have government, academic, and industry researchers from our region in attendance. This is a great way to explore potential graduate schools and employment opportunities.
2) Lunch With An Expert (Thursday lunch) – Back by popular demand, you have the opportunity to sit down with an established researcher in an area of science that interests you to discuss career opportunities and seek advice to help your with your next steps towards a career in toxicology or related STEM disciplines.
3) Laboratory Tour – If you have ever wondered what a state-of-the-art research laboratory is like to work in, here is your chance. Join us for a Laboratory Tour at WVU and you can gain some key insights and speak with graduate students, post-doc, and the laboratory boss!
4) Poster Session – Present your latest work based on your submitted abstract. Also, walk around and see what other students, graduate students, and researchers are working on. This is a great way to explore the toxicological sciences and meet others!
5) Best Undergraduate Poster Award – The second year for this award, given out to the undergraduate researcher who presents the best overall poster. A cash award and certificate will be presented Thursday afternoon. Mentors for the winning student are also recognized.
6) Informal Networking Opportunities – Besides the poster sessions, there will be plenty of time at breaks, lunch, refreshments, and Wednesday evening to chat and network with other attendees.

If you have any further questions, please contact either Dr. Stueckle or Dr. Barchowsky.

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Undergraduate Student and Mentor Resources
Society of Toxicology

The Society of Toxicology (SOT) is striving to enhance undergraduate education and improve career trajectories in the toxicological sciences. Currently, the best toxicological science draws on multiple disciplines including biology, chemistry, physics, materials science, environmental science, and more. SOT supports the teaching of toxicology to undergraduates and has an active community of undergraduate educators. The Education Committee Undergraduate Subcommittee encourages any SOT member interested in undergraduate instruction to join this Undergraduate Educator Network by subscribing in ToXchange.

The Society recognizes that Mentors are a critical component for undergraduate training, research training, and early career guidance. Resources for Mentors are available on the SOT website, including Introduction Toxicology Slide Sets, Curriculum Resources, Educator Network Webinars, and Eminent Toxicologist Lectures, which aim to introduce and integrate toxicology into your current curriculum. In addition, the ToxScholar Outreach Grant program provides travel funds to support the travel of toxicologist(s) to your school for a one or two day visit to share their career experiences and interact with students. Applications are simple! Please contact an A-ESOT representative for more information and visit the below link.
https://www.toxicology.org/education/edu/educators.asp
http://www.toxicology.org/awards/gf/toxscholar.asp

For undergraduate students, SOT offers special status as an Undergraduate Student Affiliate, specific activities at the SOT Annual Meeting, and travel awards, including the Undergraduate Diversity Award and the Pfizer SOT Undergraduate Student Award. Other resources include career information, internship listings, opportunities to participate in Regional Chapter meetings, and connections to other ways to investigate exciting opportunities in biomedical graduate school and toxicology careers.

NEW AWARD (Fall 2018): An Undergraduate Travel Award will be given to provide an undergraduate student a travel grant that would enable them to attend the Annual SOT meeting and present a poster of a Toxicology-related project. This need-based grant will ask undergraduate students to identify and work with a mentor who is an A-E SOT Member. Application materials will be submitted to the A-E SOT Leadership for the Regional Chapter to support one candidate in the national grant competition. Details will be announced soon with the first deadlines for application in September 2018.

There are a number of degree and education paths that can lead an individual to a career in toxicology. This page is designed to provide those considering a career in toxicology with information on the profession, schooling needed, and more.

https://www.toxicology.org/careers/toxicologist/becomeTox.asp

Important Yearly Deadlines for Undergraduate Activities

SOT Undergraduate Travel Award Sponsored by RC4 (NEW!)	Early September
SOT Annual Meeting Abstract Submission Deadline	Early October
SOT Awards Application Deadline	Early October
SOT Annual Meeting	Mid-March

Upcoming Meetings: **Baltimore MD – 2019, Anaheim CA 2020**
Please learn more by visiting the SOT home page, the Annual Meeting, and Undergraduate websites (see below).
https://www.toxicology.org/groups/ug/affiliates.asp
http://www.toxicology.org/events/am/AM2017/index.asp
https://www.toxicology.org/index.asp

Undergraduate Travel Award Sponsored by RC4

Purpose:

To provide an undergraduate student a travel grant that would enable them to attend the Annual SOT Meeting and present a poster of a Toxicology-related project.

Guidelines:

- This is a needs-based grant. A short 'statement of purpose' by the student will be provided clearly illustrating that the student will not be able to attend the meeting without this grant mechanism.
- The student will have to select a mentor who is a member of a Regional SOT Chapter. The student will work with the mentor on a Toxicology-related project and write an abstract.
- The application packet will include an abstract of the project, the statement of purpose, and a letter of support from the mentor, which will be submitted to the Regional Chapter.
- The Chapter will select the most meritorious candidate and put their name and application forward for further evaluation by the RC4.
- The RC4 will select the top three candidates who will receive **$1250** each.
- Partial support for the student can be provided by other sources such as the mentor or the corresponding Regional Chapter.
- The winners will participate at the SOT Undergraduate Program and present a poster of their project at the meeting. The mentor will sponsor the abstract of the undergraduate student for submission before deadline.

Tentative Timeline:

- Submission and assessment by Regional Chapter **4/1/2018 - 9/1/2018**
- Submission and Selection by RC4 Regional Group **9/1/2018 - 10/1/2018**
- Abstract submission **8/15/2018 - 10/9/2018**


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SPEAKER BIOGRAPHIES

James P. Luyendyk, Ph.D.
Dept. of Pathobiology and Diagnostic Investigation
Institute for Integrative Toxicology
Michigan State University
East Lansing, MI

Dr. James Luyendyk received his bachelor's degree in biochemistry from Colorado State University and his PhD in Pharmacology and Toxicology from Michigan State University, after which he conducted post-doctoral studies at The Scripps Research Institute. Prior to joining the faculty at Michigan State University in 2012, he was a faculty member of the Department of Pharmacology, Toxicology and Therapeutics at University of Kansas Medical Center for 5 years. Dr. Luyendyk has published more than 90 peer-reviewed research articles and reviews in the areas of liver disease and toxicity. His current research focuses on processes whereby the blood coagulation cascade is activated by hepatic injury and the mechanisms whereby coagulation proteases and their targets, namely fibrinogen, contribute to the pathogenesis of liver toxicity and repair. He is currently a member of the editorial board of *Toxicological Sciences* and *Journal of Thrombosis and Haemostasis* and serves on the NIH XNDA Study Section. Dr. Luyendyk is an active member of the Society of Toxicology, having served previously on the Education Committee and Graduate Education Subcommittee, as President of the Michigan Regional Chapter, and currently as Co-Chair of the Committee on Diversity Initiatives and Junior Councilor for the Mechanisms Specialty Section.

Contact information:

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Justin Colacino, Ph.D., MPH, MA

John G. Searle Assistant Professor of Environmental Health Sciences
Assistant Professor, Environmental Health Sciences
Assistant Professor, Nutritional Sciences
University of Michigan
Ann Arbor, MI

Dr. Justin Colacino is the John G. Searle Assistant Professor of Environmental Health Sciences and an Assistant Professor of Nutritional Sciences in the University of Michigan School of Public Health. His research focuses on understanding environmental and dietary factors in carcinogenesis and cancer prevention. Specifically, the goal of his research is to characterize the environmental susceptibility of normal human stem cell populations, elucidating the etiology of sporadic cancers. Of particular interest are understanding the changes that occur at the epigenetic and transcriptional level, changes which affect not only gene expression but also how progenitor cells differentiate and divide. His research group combines wet lab bench work and bioinformatic and statistical analysis of large scale genomic and epidemiologic data sets to translate findings from *in vitro* models to the population level. In 2018, Dr. Colacino received an Outstanding New Environmental Scientist (ONES) award from the National Institute of Environmental Health Sciences to study the biological and environmental risk factors that drive racial disparities in triple negative breast cancer. Dr. Colacino is an active member of the Society of Toxicology (SOT) and the American Association for Cancer Research (AACR) and serves on the Editorial Review Board of *Environmental Epigenetics* and the Editorial Boards of *Cancer Research and Toxicological Sciences*.

Research Areas: Primary tissue culture, stem cell biology, environmental carcinogenesis, epigenetics, cancer disparities, data mining, methods for single cell genomics

Luis A. Ortiz, MD

Professor
Division of Occupational and Environmental Medicine
Department of Environmental & Occupational Health
Graduate School of Public Health
and Professor
Division of Pulmonary, Allergy and Critical Care
University of Pittsburgh
Pittsburgh, PA

I am a Board Certified Pulmonologist and a Tenured Professor in the Schools of Public Health and Medicine at the University of Pittsburgh. My research focuses on mechanisms of lung injury that lead to the development of lung fibrosis. In particular, my laboratory has contributed to this field with the development of mouse models of pulmonary fibrosis (silica and bleomycin) and most recently with the concept that bone marrow derived Mesenchymal stem cells (MSCs) are fundamental contributors to the repair of the injured lung. Similarly, my laboratory has characterized the epidemiology of environmentally induced lung disease. Since my arrival to Pittsburgh, I established alliances with grass root organizations to initiate registries and form cohort of subjects exposed to dust (mostly miners) to characterize the impact of pneumoconiosis in the communities of Western Pennsylvania. Subsequently, I contributed to the literature with studies of the prevalence and outcome of silica exposed individuals, and their response to lung transplantation.

My basic science and translational research initiatives led to the demonstration that following the systemic administration of MSC into bleomycin or silica exposed mice these cells are retained in the injured lung and ameliorated the lung injury. Subsequently, my laboratory identified that MSCs exhibit a significant paracrine activity, exerted via secretion of extracellular vesicles and preformed proteins, and regulate innate immunity in the lung. Thus, MSC produce large amounts of the anti-inflammatory cytokine IL1 Receptor antagonist and exosomes that modulate the macrophage production of TNF and inhibit T cell proliferation.

In addition, my laboratory identified data indicating that in addition to soluble mediators, MSC release endosomal vesicles (multi vesicular bodies and exosomes) containing mitochondria and micro RNAs. Thus, MSCs can establish intercellular communication to promote genetic exchange and that further contribute to the reprogram of the inflammatory response of activated macrophages in the injured lung. In addition, we are conducting pre-clinical evaluation of these extracellular vesicles in animal model of lung fibrosis. Subsequently, our studies demonstrate that MSC production of exosomes ameliorate RV function during the development of pulmonary arterial hypertension in animal models of lung fibrosis.

Currently, my laboratory is conducting investigator initiated research to support the rational of the use of MSC in fibrotic lung diseases. To that effect, we characterized the populations of patients who are receiving medical care at the Simmons Center for Interstitial Lung Disease (ILD), where I conduct my clinical service, to inform the development of a clinically relevant animal model, such as silicosis, to test the efficacy of MSC or their exosomes in supporting the regeneration potential of the epithelial stem cell niche.

ORAL PRESENTATION ABSTRACTS

COMPARATIVE ASSESSMENT OF *IN VIVO* TOXICITY INDUCED BY MULTI-WALLED CARBON NANOTUBES AND NANOFIBERS FROM U.S. FACILITIES

K. Fraser[1,2], VK Kodali[1], N Yanamala[1], T Eye[1], D Schwegler-Berry[1], S Friend[1], MM Dahm[1], MK Schubauer-Berigan[1], ME Birch[1], DE Evans[1], NQ Wu[2], G Casuccio[3], K Bunker[3], MS Orandle[1], A Erdely[1,2]

[1]NIOSH; [2]West Virginia University; [3]RJ Lee Group

Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F), known to induce inflammation, toxicity, or tumorigenesis, is a concern during production and dry powder handling. CNT/F represent a large class of materials and it is unclear if all confer similar toxicity. Our aim was to simultaneously test the pulmonary effects induced by CNT/F with variable physicochemical properties obtained from U.S. facilities. Characterization was done for seven different multi-walled CNT and two CNF selected based on nominal diameter ranging from 10-150 nm. Cytotoxicity, inflammation, and histopathology was assessed in mice 1, 7, 28, and 84 d following oropharyngeal aspiration to 4 or 40 µg of each material. Utilized doses and material preparation for *in vivo* dosing were representative to ongoing occupational exposures. Lactate dehydrogenase (LDH) activity, a marker of cytotoxicity, was dose-dependently increased in bronchoalveolar lavage fluid (BALF) resolving toward baseline by 84 d in all groups. In materials with a diameter greater than or equal to 50 nm, LDH was persistently increased. Polymorphonuclear cell infiltration (% PMN), a marker of inflammation, was increased in all materials at 1 d post-exposure to 40 µg (<50 nm: 31.1%, ≥50 nm: 37.1%). With exposure to materials less than 50 nm, PMN influx mostly resolved by 7 d while materials greater than or equal to 50 nm induced persistent inflammation (7 d: <50 nm: 10.5%, ≥50 nm: 48.9%). For complement, inflammatory gene expression in lung tissue (e.g., *Il1b, Il6, Ccl22, Cxcl*2) and protein levels in BALF (e.g. Il1b, Il6, Il5, Ccl22, Cxcl1), were elevated to a greater extent in materials with a nominal tube diameter greater than or equal to 50 nm. In contrast, microscopic evaluation of lung sections at 84 d post-exposure indicated that histopathology does not appear distinguishable between CNT/F using diameter. In conclusion, general cytotoxicity and inflammation exhibited a relationship with nominal diameter, with a threshold of sustained effects at approximately 50 nm and greater, that was dissimilar to histopathology. Ongoing research and modeling techniques will elucidate relationships between physicochemical characteristics and toxicities of various CNT/F.

(NIOSH NTRC)

IN VIVO IMMUNO-SPIN TRAPPING DETECTION OF FREE RADICALS IN CARDIAC AND HEPATIC TISSUE FOLLOWING ACUTE EXPOSURE TO ELECTRONIC CIGARETTE VAPOR

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Electronic cigarette (E-cig) use demonstrates short- and long-term adverse health effects, however mechanisms are poorly understood. Reactive oxygen species (ROS) and subsequent oxidative stress are likely to be involved, therefore we investigated the capacity of acute E-cig exposure to generate ROS in major organ tissues using DMPO (5,5-dimethyl-l-pyrroline-l-oxide). DMPO is a 'spin trap' compound that aids in detecting the presence of free radicals by covalently binding them and forming stable adducts that can be fluorescencely imaged. We hypothesize that E-cig vapor will induce oxidative stress in all major body organs. In this work, we report the findings from heart and liver.

C57Bl/6 mice were exposed to E-cig vapor (18mg nicotine; French Vanilla flavor) for either 1 hour (n=3) or 4 hours (n=3) and compared to air-exposed controls (n=3). For all groups, mice were injected with DMPO and euthanized 1.5 hours afterwards. The organs were systemically perfused with physiological buffer solution (PBS) via intracardiac injection before harvest. Immunohistochemistry was performed on organ tissue to visualize site and extent/degree of oxidative stress.

In heart, there was a 33% increase in fluorescence signal between 1-hr expose (50.92±1.7 RLU) and controls (38.36±6.8 RLU) ($p<0.05$). In liver, there was a 122% increase in the 1-hr exposed group (21.41±4.0 vs. 9.64±4.0 RLU, $p<0.05$). No significant change seen between 1-hr and 4-hr exposed groups.

Acute exposure to E-cig vapor and its bioactive constituents induced demonstrable oxidative stress in liver and heart. This reveals a potential mechanism by which E-cig usage causes adverse systemic health effects.

Funding: West Virginia School of Medicine Dean's Office and Phillip R. Dino Innovative Research Grant

ENM INHALATION DURING GESTATION DISRUPTS PLASMA ESTROGEN AND VASCULAR KISSPEPTIN REACTIVITY

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Maternal engineered nanomaterial (ENM) inhalation during gestation is associated with uterine vascular impairments and endocrine disruptions that lead to altered gestational outcomes. However, the mechanism underlying this dysfunction is unknown. One possibility is alterations in estrogen (E_2), which is critical for pregnancy and required for synthesis of the potent vasoconstrictor, kisspeptin (Kiss). Therefore, we examined how E_2 and Kiss are involved in nano-titanium dioxide (nano-TiO_2)-induced vascular dysfunction. Pregnant (gestation day (GD) 6) Sprague-Dawley rats were exposed to nano-TiO_2 aerosols (cumulative, calculated dose = 217 ± 1.0 µg; n=6) or sham exposed (n=6). Plasma was collected on GD 20 to evaluate E_2, progesterone (P_4) and corticosterone (CORT). In a separate series of experiments, virgin rats were exposed via intratracheal instillation to nano-TiO_2 P25 powder, obtained from Evonik, (100 µg) suspended in 200 µL of vehicle (normosol and 5% fetal bovine serum, n=7) or sham controls (n=5). 24 hours later thoracic aorta was dissected, and 2-3 mm segments were cut and mounted in wire myograph chambers (DMT 620M) containing physiological salt solution (PSS) at 37°C. After 1 hr of equilibration, maximum contractile response was determined using high-potassium PSS. Vascular segments were washed with PSS until initial tension returned. Contractile responses were determined *via* addition of one 50 µL dose of phenylephrine ($1x10^{-2}$) and cumulative 50 µL doses of Kiss-10 (1×10^{-8} to 1×10^{-3} M). Plasma E_2 was decreased at GD 20 in exposed versus control rats (11.08±2.5 vs. 66.97±2.5 pg/mL; $p<0.05$), whereas there were no differences in P4 or CORT. Kiss1 increased aortic tension in exposed animals compared to controls (142%±6 vs.108±7%). These studies represent the first evidence pulmonary ENM exposure perturbs the normal gestational endocrine vascular axis via a Kiss dependent mechanism.

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ARSENIC DISRUPTS MUSCLE STEM CELL DETERMINATION THROUGH FIBROBLAST MITOCHONDRIAL MALADAPTATION THAT DIRECTS A DYSFUNCTIONAL EXTRACELLUAR MATRIX MEMORY

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Chronic arsenic (As(III)) exposure increases risk of adverse health outcomes that include skeletal muscle dysfunction and mobility decline. We reported that As(III) impairs muscle maintenance and regeneration by inducing maladaptive mitochondrial phenotypes in muscle stem cells (MuSC), connective tissue fibroblasts (CTF), and fibers. We also found that As(III) imparts a dysfunctional memory in the extracellular matrix (ECM) that disrupts the MuSC niche and is sufficient to misdirect differentiation of muscle progenitor cells from myogenesis to fibrogenesis. Therefore, we asked whether dysfunctional mitochondria in As(III)-exposed CTF directed ECM alteration and whether restoring CTF mitochondrial function reverts the ECM memory. CTF were isolated from hind limb muscles of mice exposed to 0 or 100 µg/L As(III) in drinking water for 5 weeks. The CTF and elaborated ECM for 2 days before decellularization. Human muscle progenitor cells (HMPC) were seeded onto the elaborated ECM and differentiated for 2-3 days. There were fewer properly formed multinucleated myotubes from HMPC seeded on ECM derived from As(III)-exposed CTF, relative to control. In addition, MyoD and desmin, indicators of myogenic differentiation, were downregulated in the cells derived from HMPC plated on As(III)-CTF ECM at the same time cells expressing PDGFRα and CD34, indicators of fibroblast/adipocyte progenitors, were increased. To demonstrate that As(III)-impaired mitochondrial function is responsible for CTF elaborating a pathogenic ECM, we treated mice with SS-31, a peptide that repairs inner mitochondrial matrix architecture, for 1 week after the 5 week As(III) exposure period. Selective injury to the tibialis anterior (TA) muscle with BaCl2 followed by 2 week recovery examined the restorative effects of SS-31 on muscle regeneration. SS-31 restored arsenic-impaired TA regeneration and HMPC seeded onto ECM elaborated by CTFs isolated from As(III)-exposed/SS-31-treated mice had normal myotube differentiation and MyoD expression, as well as increased desmin expression, compared to ECM of CTF from control or As-exposed mice. These data indicate that As(III) impairs muscle maintenance and regenerative capacity by targeting CTF mitochondria.

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MATERNAL PULMONARY EXPOSURE TO TITANIUM DIOXIDE NANOPARTICLES ALTERS PLACENTAL HEMODYNAMICS

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The increasing and widespread integration of engineered nanomaterials in domestic and commercial applications is well-established. Reproductive and cardiovascular dysfunction have been observed in adult populations, however the fetal consequences of maternal exposure have yet to be fully understood. The placenta is one of the critical protective barriers providing protection to the unborn fetus and allowing the continuous transfer of nutrients, and hormones from the maternal circulation. The purpose of this study was to determine the effects of pulmonary titanium dioxide nanoparticle (nano-TiO_2) exposure on placental hemodynamics. We hypothesized that pulmonary nano-TiO_2 exposure disrupts placental hemodynamics by increasing vascular resistance. Female Sprague-Dawley rats were exposed via whole-body inhalation to nano-TiO_2 with a primary particle size of 21 nm, specific surface area of 48.08 m^2/g, and Zeta potential of −56.6 mV. Animals were exposed on GD 11 for 7 days (4-6h/exposure) to achieve a daily calculated lung deposition of 31 ± 1.1 µg per day, and a cumulative, calculated dose of 217 ± 1.0 µg. Placentae were isolated, cannulated via the umbilical artery and vein on GD 20, and treated with acetylcholine (ACh), angiotensin II (ANGII), kisspeptin (KISS), S-Nitroso-N-acetyl-DL-penicillamine (SNAP), or calcium-free superfusate. Mean outflow pressure and flow rate were measured in the isolated placental units. Nano-TiO_2 exposure was associated with a significant decrease ($p<0.05$) in mean outflow pressure in pre-treated placentae, as well as those treated with ACh and calcium-free superfusate. At 80 mm Hg inflow pressure, mean outflow pressure in pre-treated control placentae was 42.78 ± 1 mm Hg, while those from exposed animals were 31.55 ± 3.4 mm Hg. ACh-treated placentae presented mean outflow pressures of 52.99 ± 4.5 mm Hg in controls and 34.52 ± 4.4 mm Hg in exposed subjects. Lastly, calcium-free superfusate yielded outflow pressures in control and exposed rats of 62.58 ± 4.7 mm Hg and 29.42 ± 9.5 mm Hg respectively. These results suggest that gestational pulmonary nano-TiO_2 exposure alters placental hemodynamics by potentially increasing vascular resistance via disruption of endothelium-dependent dilation or by inducing structural changes within the placenta.

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BROCCOLI-DERIVED SULFORAPHANE PREVENTS FORMATION OF MAMMARY TUMORS IN RATS EXPOSED TO 17B-ESTRADIOL

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Elevated levels of estrogens have been recognized as an important determinant of breast cancer risk. The estrogen receptor-independent, oxidative metabolism of estrogens is an important pathway for estrogen-mediated carcinogenesis. Studies have shown that select estrogen metabolites can form depurinating estrogen-DNA adducts leading to mutations and cancerous lesions. In healthy cells, the formation of these DNA-damaging mutations are controlled by multiple cytoprotective enzymes such as catechol-*O*-methyltransferase (COMT), glutathione-*S*-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1). Pharmacologic activators of the Nrf2 signaling pathway such as broccoli-derived sulforaphane have been shown to activate these enzymes. Therefore, we hypothesized that administering sulforaphane to animals that are exposed to17β-estradiol would prevent mammary tumor formation via reduced formation of depurinating estrogen-DNA adducts. In our study, 4-6 week old female August Copenhagen Irish rats were implanted with 17β-estradiol pellets and were simultaneously gavaged with either DMSO or 100 µmol/kg sulforaphane for 56 weeks. The Kaplan-Meir curve showed that sulforaphane treated rats were significantly protected against mammary tumor formation compared to DMSO controls. While sulforaphane-treated rats were protected against mammary tumor formation, once tumors formed, they exhibited a more aggressive phenotype. Further characterization also suggested that sulforaphane treatment may alter the genetic landscape of the mammary gland as well as change the metabolic profile of these rats.

This study was funded by the Breast Cancer Research Foundation and the National Institutes of Health (R35 CA197222).

GHS HAZARD INVERSION TECHNIQUES; MEASURING BIOAVAILABILITY

Don Ewert

Occupational Health Services, RJ Lee Group INC, Monroeville, PA

With adoption of the Globally Harmonized System of Classification and Labeling (GHS-2015), OSHA has incorporated a highly complex characterization scheme into what was once, a well-structured system of Hazard Communication. Given a new allowance for “expert judgement”, however, OSHA’s “Letter-of-Interpretation” library is destined to swell. All because authors can now choose between an ingredient approach and final mixture paradigm. While the first method offers simplicity and processing speed, the latter requires rigor and a host of analytical techniques. Both methods have advantages, but only the final mixture approach offers Hazard Inversion on a scientifically validated basis.

VIRAL-MEDIATED DYSREGULATION OF VESICULAR MONOAMINE PACKAGING IS TOXIC TO DOPAMINERGIC NEURONS

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Within dopaminergic (DAergic) neurons, dysregulation of dopamine (DA) packaging results in increased cytosolic DA, which is susceptible to oxidation and degradation: two processes that generate reactive metabolites and reactive oxygen species. Cytosolic DA can result in neurodegeneration (Caudle et al. 2007, Chen et al. 2008), however, the sequestration of DA by the vesicular monoamine transporter 2 (VMAT2) prevents DA from acting as an endogenous neurotoxin by removing it from the cytosol. To understand the mechanisms of cytosolic DA-induced neurotoxicity, we constructed a viral vector (AAV2-shVMAT2) to decrease VMAT2 expression by small-hairpin ribonucleic acid interference. Adult rats received unilateral injections of AAV2-shVMAT2 in the nigrostriatal DAergic pathway, targeting neurons with cell bodies in the substantia nigra (SN) and terminals in the striatum. These neurons are the degenerating neurons in Parkinson's disease (PD), and there is significant evidence that deficits in vesicular packaging of DA contribute to the pathogenesis of PD (Miller et al. 1999, Pifl et al. 2014). Following AAV2-shVMAT2, VMAT2 protein expression is decreased by 36.6% in transduced SN neurons and by 44% in the striatal terminals (paired t-test, $n=5$, $p<0.05$). There was a corresponding loss of DA by 49.6%, increased DA turnover by 64.6%, and increased DA oxidation by 27.5% (paired t-test, $n=4$, $p<0.05$). The dysregulation of DA packaging resulted a loss of DAergic neurons in the SN by 38.7% (paired t-test, $n=5$, $p<0.05$), and decreased DAergic terminal staining in the striatum by 29.8% (paired t-test, $n=5$, $p<0.05$). These results demonstrate that viral-mediated targeting of VMAT2 can be used to dysregulate DA packaging, thereby generating a model by which cytosolic DA mediated toxicity can be studied.

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ENVIRONMENTAL MITOCHONDRIAL TOXICANTS AND LRRK2; A NEW GENE-ENVIRONMENT INTERACTION IN PARKINSON'S DISEASE

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Environmental toxicants that cause mitochondrial dysfunction, such as the organic pesticide rotenone, and the common herbicide paraquat, are associated with elevated PD risk. A heavily used industrial solvent, trichloroethylene (TCE), also causes mitochondrial toxicity, and is a ubiquitous environmental contaminant. Occupational TCE exposure is linked to the development of PD, and rodent models of using high doses of TCE display dopaminergic neurodegeneration.

New data from our lab indicates that rotenone, paraquat, and TCE interact with PD susceptibility genes, notably, causing the activation of the protein LRRK2 (leucine-rich repeat kinase 2) in wildtype human embryonic kidney (HEK) cells, which could be blocked by a selective LRRK2 inhibitor (GNE-7915). As LRRK2 is the most commonly inherited mutation associated with familial PD, this evidence suggests that *a gene-environment interaction exists between LRRK2 and common environmental mitochondrial toxicants*. Functionally, LRRK2 activation leads to pleiotropic cellular dysfunction, such as disruption of vesicular trafficking and autophagy, accumulation of phosphorylated α-synuclein, and neuroinflammation; all of which are mechanisms hypothesized to precede dopamine neuron degeneration in PD.

As TCE is a widespread environmental contaminant, we postulated that relatively low levels of TCE exposure in aged rats may induce LRRK2 activation in dopaminergic neurons and increase risk of a parkinsonian phenotype. In a pilot study, we exposed adult, male Lewis rats (7-9 mo.) to 200 mg/kg of TCE (ingested) or vehicle for 45 days. Animals receiving TCE displayed a moderate loss of dopaminergic neurons, which correlated with a significant increase in LRRK2 kinase activity in surviving cells. In addition, we observed deficits in endolysosomal trafficking, concomitant with accumulation of α-synuclein, suggesting that LRRK2 activation by TCE may be a novel gene-environment interaction that increases PD risk.

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MEASUREMENT OF TELOMERE LENGTH AND REGULATORY GENES IN CIRCULATING PERIPHERAL BLOOD MONOCYTES AND LUNG TISSUE AS A BIOMARKER FOR RESPONSE AFTER INHALATION EXPOSURE TO OCCUPATIONAL PARTICLES

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The measurement of telomere length may serve as a potential biomarker to assess occupational exposure. Telomeres are simple repetitive structures of DNA sequences $(TTAGGG)_n$ that stabilize ends of chromosomes by preventing DNA degradation and preserving genetic information. Telomeres generally shorten with age, and their length may be influenced by stressors, such as occupational exposures. The goal of the study was to measure telomere length and genes involved in regulation (e.g., regulator of RTEL1 and TERT) in PBMCs and in lung tissue after exposure to different workplace particles in an animal model. Using different exposure paradigms, whole blood, PBMCs and lung tissue were collected from male Fischer-344 rats: (1) at 1 d and 12 wk after inhalation to 20 mg/m^3 x 3 hr/d x 4 d/wk x 5 wk of stainless steel welding fume (SS-WF) and (2) at 4, 32, and 44 wk after inhalation to 15 mg/m^3 x 6 h/d x 5 d of silica (Si). In both paradigms, control animals were exposed to filtered air. SS-WF inhalation caused a significant decrease in telomere length in circulating PBMCs and in lung tissue over the 12 wk time course compared to control. On the other hand, telomere length was significantly increased in lung tissue in the Si-exposed group at 4 and 32 wk compared to control. Importantly, RTEL1 expression was significantly decreased in lung tissue of the Si-exposed animals at all-time points compared to control, whereas TERT expression was significantly increased at 4 and 32 wk. Thus, varied responses in regards to telomere length and regulation were observed after different occupational exposures and were specific for the agent. Telomere length and expression of telosome and regulatory genes may serve as potential biomarkers related to occupational exposure and may offer insight into the molecular mechanism of particle-induced lung disease.

POSTER ABSTRACTS


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M Shoeb	NIOSH	39 (YI)
M Shoeb	NIOSH	40 (YI)
GM Mustafa	NIOSH	41 (YI)
TA Stueckle	NIOSH	42
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ANALYSIS OF SITAGLIPTIN IN MILK SAMPLES USING GAS CHROMATOGRAPHY MASS SPECTROMETRY

B Day, HN Currie

Frostburg State University, Frostburg, MD

Januvia, the brand name of Sitagliptin (STG), is prescribed to patients managing type II diabetes mellitus. While effective in treating type II diabetes, STG can cause significant and harmful side effects including pancreatitis and kidney damage. These side effects can even lead to death. A study in rats showed STG is secreted in the milk of lactating rats, but to our knowledge, there have been no studies of STG conducted on breastfeeding women. Even though there is little research regarding STG and breastfeeding, it is still prescribed to breastfeeding women. The aim of this research was to develop a method to detect STG in milk by extracting the STG, derivatizing it with MSTFA, and analyzing it with gas chromatography mass spectrometry (GC-MS). A standard curve was created and a known sample of cow's milk was spiked with 156 ppm STG. The developed GC-MS method determined the concentration of the spiked sample to be 151 ppm. Future studies are under way to use the developed method to test the milk of breastfeeding women that were prescribed STG.

This research was funded by the Provost's Experiential Learning Enhancement Program and the Chemistry Department at Frostburg State University.

CONDITIONALLY DEPENDENT PRODUCTION OF TOXIC ERGOT ALKALOIDS BY FUNGI IN THE GENUS *METARHIZIUM*

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Ergot alkaloids are important agricultural toxins. Derivatives of ergot alkaloids are used as pharmaceuticals to treat dementia, migraines, and hyperprolactinemia. Publicly available genomic sequence data indicate that fungi in the genus *Metarhizium* have the capacity to produce lysergic acid-derived ergot alkaloids; however, accumulation of ergot alkaloids in these fungi has not been demonstrated previously. *Metarhizium* species colonize soil, roots of many plants, and insects. For these reasons, we investigated the capacity of *Metarhizium* species to produce these important environmental chemicals. We assayed several *Metarhizium* species grown under different conditions for accumulation of ergot alkaloids by high performance liquid chromatography with fluorescence detection. *Metarhizium flavoviride* did not accumulate ergot alkaloids on any of three culture media, but *Metarhizium anisopliae* accumulated large quantities of the ergot alkaloids lysergic acid α-hydroxyethylamide (LAH), ergine, ergonovine, and chanoclavine-I on sucrose yeast extract agar, lesser quantities on malt extract agar, and none on corn meal agar. The identities of the alkaloids were confirmed by mass spectrometry. Interestingly, *M. anisopliae* secreted over 80% of its alkaloid yield into the medium, whereas ergot alkaloids of other fungi are retained in their hyphae. We inoculated roots of corn (*Zea mays*), bean (*Phaseolus vulgaris*), and *Medicago truncatula* with *M. anisopliae* and *M. flavoviride*, and no ergot alkaloids were produced by either fungus on any plant. Four of five tested *Metarhizium* species produced high concentrations of ergot alkaloids in infected larvae of the model insect *Galleria mellonella*. The data demonstrate that several *Metarhizium* species produce ergot alkaloids of the lysergic acid amide class and that production of ergot alkaloids is tightly regulated and associated with insect colonization.

THE FIBROTIC POTENTIAL OF NANOCLAY IN MICE

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Objective: The aim of this study was to determine the protein expression of markers of reticular fiber fibrosis in the lungs (collagen III and fibronectin) following exposure to non- and incinerated organomodified nanoclay in mice.

Introduction: Nanoclay is derived from soil clay with organic coating modification to achieve desirable properties. Through incorporation of nanoclay into polymeric matrixes, the nanoclay composite acquires enhanced properties like increased mechanical and thermal strength, enhanced physical and barrier properties that support its utilization in many industries like automotive, medical device, construction and food industries. With increased use of nanoclay composite, increased airborne occupational ONC exposure to workers is expected to increase with potential adverse health effects.

Method: C57BL/6J mice were exposed to high doses (300 µg) of incinerated and non-incinerated Cloisite Na^+ (Uncoated (UC-900) and UC) and organically modified nanoclay Cloisite 30B (Coated (CC-900) and CC) for 28 days and lung tissue was assessed for markers of reticular fiber fibrosis. The markers assessed were fibronectin and collagen III through immunohistochemistry. Saline and silica exposed mice were used as negative and positive controls, respectively. All animal exposure through oropharyngeal aspiration and tissue processing were done by NIOSH in West Virginia.

Result: There is increased expression of fibronectin in the uncoated nanoclay exposed mice compared to the coated nanoclay-exposed mice. In addition, there is also increased expression of collagen III in the uncoated nanoclay-exposed mice compared to the coated nanoclay-exposed mice. Our data suggests that incineration of nanoclay decreases the expression of fibronectin and collagen III in exposed mice compared to non-incinerated nanoclays.

Conclusion: The results suggest that coating and incineration status of nanoclay play a role in the expression of collagen III and fibronectin in mice. More studies need to be done to establish this and possibly translate to the fibrotic effect in humans.

Funding: Nanotechnology Research Center, NIOSH

DETERMINATION OF ANTIOXIDANT STRENGTH VIA HORSERADISH PEROXIDASE CATALYZED BIODEGRADATION OF SINGLE-WALLED CARBON NANOTUBES

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Antioxidants have long been known for their ability to inhibit oxidation of various molecules, by scavenging free radicals, in the human body. Excess formation of free radicals in cells can cause membrane or tissue damage, break DNA strands, and initiate biological responses leading to cancer, arthritis and other inflammatory diseases. Thus, antioxidants could play a key role in diseases associated with oxidative stress. However, successful development of effective antioxidant therapies remains a debate, as the ability of antioxidants, in particular secondary antioxidants, to inhibit oxidation depends on various factors including their function, mechanism of action and classification. Antioxidants are commonly classified as primary antioxidants, secondary antioxidants, chelators, quenchers, oxygen scavengers, and antioxidant regenerators/synergists. In this study, a set of antioxidants including α-Tocopherols – a primary antioxidant and a synergist; β-Carotene – a quencher; Glutathione - a cell-synthesized antioxidant with a thiol group; and Melatonin - one that does not undergo redox cycling, were selected to test and compare their ability to inhibit an enzyme-driven degradation of oxidized carbon nanotubes (CNTs). In this approach, horseradish peroxidase-generated oxidants, in the presence of H2O2, are the reactive species that fulfill the biodegradation of CNTs, where the color of solution gradually changes from black to clear. The ability of an antioxidant to inhibit this process was monitored overtime (~21 days). Of all the antioxidants considered in the study, α-Tocopherol was the most efficient in inhibiting or mitigating the degradation of the oxidized CNTs over the same time periods. Mechanisms that could be responsible for such mitigation may include differences in antioxidants (a) redox potentials, (b) reduction of functional groups on nanomaterial surface, which are necessary for enzyme-catalyzed degradation, and (c) direct interaction with peroxidase with or without interfering with the binding site that is occupied by SWCNT. To further understand and differentiate between the interactions of various antioxidants selected, molecular docking studies were performed to gain insights into the molecular details of their interaction with peroxidase.

HIGH THROUGHPUT *IN VITRO* TOXICITY SCREENING OF AS-PRODUCED NANOCLAYS USED IN NANOCLAY-ENABLED COMPOSITES

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The utilization of organomodified nanoclays (ONCs) in manufactured composite materials is expected to rapidly expand into a $3.37 billion global market by 2023 due to the conferred moisture barrier properties, tensile strength, and thermal stability nanoclay adds to various composites. The scientific literature lacks data on aerosolized particle release from ONC-enabled composite during machining (i.e. sanding) as well as potential to cause adverse pulmonary effects. Previous ONC pulmonary exposures in mice demonstrated varied cytotoxic, inflammatory, and pro-fibrotic responses dependent on the presence of a quaternary ammonium compound (QAC) coating. The objective of this study was to 1) evaluate several toxic endpoints in multiple lung cell types via a high throughput *in vitro* screening system following Cloisite Na, Cloisite 25A, and Cloisite 93A exposure, and 2) evaluate particle characteristics released from 4% ONC-enabled polypropylene composite following controlled machining activities. As-produced ONC was first characterized in liquid suspension with FESEM and dynamic light scattering, then exposed *in vitro* to THP-1 macrophages and human small airway epithelial cells (hSAEC) at doses from 0.6 -20 µg/cm^2 to assess cell viability, caspase-3 expression, reactive oxygen species (ROS) levels, and Cathepsin B release from lysosomes. ONC suspended in immersion culture with bovine lung surfactant (Survanta) showed the most stability in the submicron size range for all tested particles. Initial *in vitro* screening results demonstrate dose-dependent cytotoxicity, caspase-3 expression, ROS, and Cathepsin B release in exposed THP-1 macrophages and hSAECs, where the severity of each is modulated by the presence or type of QAC coating. Additionally, 4% nanoclay-enabled polypropylene was synthesized, sanded in a simulation chamber with real time particle counts, and released particles were characterized with FESEM and TEM. Electron microscopy imaging coupled with real time particle counts during composite sanding show preliminary evidence for the release of inhalable and respirable composite fragments with protruding ONC. Combined, this data illustrates the potential for occupational pulmonary exposure following machining of ONC-enabled composite and subsequent airway cytotoxicity as a result of ONC exposure.

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PROPANIL ACUTELY ALTERS REPRODUCTIVE HORMONES AND IMMUNE CELL POPULATIONS IN HEAT-KILLED *STREPTOCOCCUS PNEUMONIA* EXPOSED FEMALE MICE

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Propanil is a post-emergent herbicide used on rice and wheat fields. It has previously been demonstrated that propanil exposure (200mg/kg) increases the number of antibody-secreting cells in the spleen after heat-killed *Streptococcus pneumoniae* (HKSP, $2x10^8$ CFU) immunization. The hypothalamic-pituitary-gonadal axis has been demonstrated to be required for this response in female, but not male mice. As a result, it was hypothesized that propanil will alter progesterone, prolactin and estradiol levels, as well as modify immune cell populations. To test this hypothesis, 24 C57Bl/6 female mice were challenged with HKSP and assigned to four treatment groups (6 mice each) in a 2x2 factorial arrangement. Factor one represented propanil treatment (propanil, control). The second factor represented two collection time points (24h, 72h). Serum progesterone, prolactin, and estradiol concentrations were measured using hormone-specific ELISA assays. Splenic T cells ($CD4^+$ and $CD8^+$), B-cells and $CD11b^+$ populations were analyzed by flow cytometry. Twenty-four hours post-propanil exposure and HKSP immunization, progesterone (p=0.0311) and prolactin (p=0.0167) levels were significantly increased in comparison to HKSP immunized controls. Estradiol levels were not affected. Propanil decreased the total number of splenocytes (p=<0.0001), $CD8^+$ T cells (p=0.0012), $CD11b^+$ cells (p=0.0022) and B cells (p=0.0003). The percentage of $CD8^+$ T cells (p=0.0001) and $CD11b^+$ cells (p=0.0022) were increased, while the percentage of B cells (p=0.0003) decreased. No significant effects were noted 72 hrs post-propanil exposure. Future studies will investigate the mechanism by which propanil alters hormone levels and immune cell populations.

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CLASSIFICATION OF CHEMICAL HYPERSENSITIVITY POTENTIAL BASED ON GENE EXPRESSION PROFILES

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Occupational allergic disease is a serious public health burden that can result in asthma and allergic contact dermatitis and is often a result of exposure to low molecular weight chemicals. The classification of chemical allergens has proven to be difficult since many agents can elicit multiple hypersensitivity pathways. Additionally, studies are also demonstrating a role for exposure to non-sensitizing chemicals such as adjuvants and irritants in allergic disease. However, the complete immunological mechanisms driving these responses are not fully understood.

It is increasingly being recognized that the skin plays a major role in the development of allergy due to its complex immunological environment. Preliminary data from our lab and others have shown that cells in the skin can secrete a variety of cytokines and molecules in response to chemical exposure, supporting an important role in immunological responses.

For these studies, BALB/c mice were dermally exposed to representative chemicals (sensitizers, irritants, adjuvants) and the changes in gene expression of cytokines and cellular mediators was evaluated in skin, draining lymph nodes, and blood over a period of 7 days. Results identified unique gene expression profiles for select cytokines and molecular mediators. Following exposure to the adjuvant, *A20* and *TLR4* were uniquely increased in the skin. Exposure to the sensitizers resulted in expected changes in cytokine expression in the lymph node along with unique increases in *IL-10* and decreases in *A20* in the blood. Additionally, increases in *S100A8* expression was observed in the blood following exposure of select sensitizers and irritants.
In order to further the development of preventative and therapeutic strategies to combat allergic disease, the underlying mechanisms must be fully understood. Defining unique gene expression profiles could allow us to narrow in on immunological pathways to help with the identification and classification of chemical allergens while optimistically leading to the discovery of novel mediators.

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SURFACE AREA- AND MASS-BASED COMPARISON OF LUNG TOXICITY AND ALLERGIC EXACERBATION IN AN OVALBUMIN ALLERGY MODEL FOLLOWING PULMONARY EXPOSURE TO FINE AND ULTRAFINE NICKEL OXIDE

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The correlation of specific nanomaterial physico-chemical properties with toxicological responses is an area of growing interest. In this study, the role of nickel oxide mass and surface area in the induction of pulmonary inflammation and exacerbation of respiratory allergy was explored. To address this concept, 181 nm fine (NiO-F) and 42 nm ultrafine (NiO-UF) particles were characterized and incorporated into an *in vivo* time course study and ovalbumin (OVA) asthma model. Particle toxicity was compared at equal masses of 40 µg and at equal surface areas of 1.92 mm^2. For the time course study, female BALB/c mice were exposed once to particles or vehicle control by oropharyngeal aspiration and euthanized 1, 10, 19, or 29 d post-exposure, which represent critical time points in the OVA model. For the OVA model, mice were aspirated with particles on d 0, sensitized to OVA via IP injection on d 1 and 10, challenged with OVA by aspiration on d 19 and 28, and euthanized on d 29. In the time course study, exposure to mass-normalized doses of particles resulted in significantly elevated LDH levels, lung neutrophils, and mediastinal LN size in mice exposed to NiO-UF, which persisted to 29 d post-exposure. However, normalization of doses for surface area mitigated all differences between particles, suggesting that NiO surface area drives pulmonary inflammation. In the OVA model, exposure to NiO, irrespective of particle size, dose mass, or surface area resulted in elevated circulating total IgE levels over allergy control animals. However, elevations in OVA-specific IgE were correlated to NiO dose surface area in addition to lung neutrophils and BALF cytokine profiles. Penh and lung eosinophil number appeared correlated with NiO particle size and interestingly, BALF IL-6 levels were conserved among groups exposed to a 40 µg dose of either particle. Overall, findings suggest that although surface area of NiO dictates pulmonary injury and inflammation, it may not be the only physico-chemical property responsible for modulation of immune responses in the lung.

TOXICITY ASSESSMENTS OF NANOCLAY SYSTEMS THROUGHOUT THEIR LIFE CYCLE

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Montmorillonite nanoclays functionalized with organic modifiers can be incorporated in polymers to form nanocomposites with enhanced mechanical, barrier, and UV dispersion properties to be applied in areas such as food packaging. However due to their small size and increased reactivity, nanoclays and associated byproducts have the potential to be inhaled because of worker inhalation hazards during their life cycle. Evaluating their potential to induce deleterious effects in biological systems is thus required in order to create proper safety protocols for both manufacturing and disposal.

Herein, we investigated the material properties and toxicity of the ‘as-received’ nanoclay, Cloisite 30B (functionalized with methyl, tallow, bis-2-hydroxyethyl, quaternary ammonium) and its thermally degraded byproduct, used to mimic the end of life cycle, disposal stage via the route of incineration. Further, the material properties of a polylactic acid-Cloisite 30B nanocomposite was investigated, along with the toxicity of its thermally degraded byproduct. Human bronchial epithelial (BEAS-2B) cells were used to model inhalation toxicity with electric cell-substrate impedance sensing being used to provide cellular behavior analyses in a real-time and high throughput manner. Both Cloisite 30B and the thermally degraded byproducts induced cellular changes, including reductions in cell viability, changes in cellular morphology, and cytoskeletal alterations. Further, Cloisite 30B in its ‘as-received’ form displayed the highest degree of toxicity relative to its byproduct and the nanocomposite byproduct, likely due to the presence of its organic modifier. Our results show that the materials’ properties throughout the nanoclay and nanocomposite life cycle could lead to cellular toxicity and hint at the need to implement safety norms for doses mimicking both acute and sporadic exposure.

LACK OF LUNG TUMOR PROMOTION AFTER INHALATION OF A COPPER-NICKEL WELDING FUME IN A/J MICE

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The International Agency for Research on Cancer classified welding fumes as a Group 1 carcinogen (*carcinogenic to humans*) in 2017. The process of stainless steel welding creates fumes rich in carcinogenic metals such as chromium (Cr). Our lab has previously demonstrated that stainless steel welding fumes promote lung tumors in tumor-susceptible A/J mice. Consumables devoid of Cr are being produced in an attempt to limit worker exposures to potentially carcinogenic metals. The aim of this study was to characterize a new copper-nickel (Cu-Ni) fume and then investigate if inhalation of this fume would promote lung tumors in mice using a two-stage (initiation-promotion) model. To determine particle mass size distribution, a Micro-Orifice Uniform Deposit Impactor (MOUDI, model 110; MSP corp., Shoreview, Minn.) with additional Nano-MOUDI stages (MSP model 115) was used. Characterization of the fume indicated that most of the particles were between 0.1 and 1 µm in diameter, with a mass median aerodynamic diameter of 0.43 µm. Male A/J mice (4 – 5 weeks old) were initiated with 3-methylcholanthrene (MCA; 10 µg/g IP) or corn oil and, beginning 1 week later, were exposed to air or Cu-Ni welding fumes for 4 hours/day, 4 days/week, for 9 weeks. At 30 weeks, mice were sacrificed and lung tumor multiplicity and incidence were evaluated. MCA/Cu-Ni welding fume exposure significantly decreased tumor size and tumor number compared MCA/air controls (7.11 ± 0.93 tumors *vs*. 15.57 ± 0.75 tumors and 0.57 ± 0.01 mm in diameter *vs*. 1.15 ± 0.02 mm in diameter, respectively). Future studies are planned to investigate the pneumotoxicity of Cu-Ni fume in A/J mice.

CONSEQUENCES OF MATERNAL ENGINEERED NANOMATERIAL INHALATION ON FETAL VASCULAR REACTIVITY

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Nano-TiO_2 is a widely used engineered nanomaterial (ENM). Although nano-TiO_2 is frequently utilized, the vascular health ramifications of maternal-fetal exposure during gestation are poorly understood. The aim of this study was to determine the fetal vascular consequences of maternal inhalation exposure. Pregnant Sprague-Dawley rats were exposed to aerosolized nano-TiO_2 and vascular reactivity of fetal aortic rings was measured. The experimental group details included: dam age 100$\pm$1 days, dam weight 373$\pm$17 g, litter size 13$\pm$1 pups, and pup weight 4.0$\pm$0.1 g. The bulk material, Evonik-P25, had a primary particle size of 21 nm and zeta potential of -56.6 mV. Inhalation was performed in a whole-body exposure chamber for 6 hours per day. Mass concentration (10$\pm$0.5 mg/m^3) and size distribution of nano-TiO_2 was monitored. The mean aerodynamic diameter was 139 nm and mobility diameter was 153 nm. Rats were exposed to aerosolized nano-TiO_2 beginning at gestation day 10 for 7-8 days. The calculated lung deposition was 217 μg which reflects a human lung deposition of 55 mg. The Occupational Safety and Health Administration (OSHA) exposure limit for nano-TiO_2 is 2.55 mg/day. This parameter would allow for our calculated lung burden to be achieved in 22 working days in humans. This represents a highly relevant exposure model as human gestation is 9 months. The dams were sacrificed 24 hours after the final exposure and fetal aortas were dissected and prepared for wire myography. Fetal aortic ring tension generation was assessed using cumulative concentrations of the prostaglandin H_2 analog U46619 ($1x10^{-14}$--^{7} M), endothelium-dependent agonist acetylcholine ($1x10^{-9}$--^{4} M) and endothelium-independent agonist S-Nitroso-N-acetylpenicillamine ($1x10^{-9}$--^{4} M). These results represent the initial evidence that fetal aortic reactivity is altered after maternal nanomaterial inhalation.

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DISCOVERY OF PSYCHOACTIVE COMPOUNDS CATHINONE AND PSILOCYBIN IN THE CICADA PATHOGEN *MASSOSPORA*

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The obligate lifestyle and ephemeral nature of Entomophthoralean fungi have long stymied in vitro studies. Recent ex vivo expression and metabolomics studies involving entomopathogenic fungi have provided unprecedented functional insight, yet most remain unexplored. Here we show that global and targeted metabolomics unexpectedly revealed two biologically significant monoamine alkaloids, cathinone, a plant-based amphetamine, from *Massospora cicadina*-infected periodical cicadas and psilocybin, a mushroom-derived tryptamine, from *M. platypediae*-infected annual cicadas and archived *M. levispora* specimens. The known biological activity of these alkaloids provides a hypothetical framework for an “extended behavioral phenotype” resulting from *Massospora* infections. These psychotropic metabolites likely enhance promiscuity while suppressing feeding by cicadas to maximize conidial dispersal before death. Together, metabolomics, metagenomics, and protein expression results provide strong evidence for alkaloid biosynthesis in *Massospora*.

(West Virginia Agricultural and Forestry Experiment Station, Protea Biosciences)

XOR-Catalyzed Nitric Oxide Generation: Implications for Toxicant-Mediated Inflammatory Response

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The capacity of molybdopterin enzymes to reduce nitrite (NO_2^-) to nitric oxide ($^{\bullet}NO$) is increasingly appreciated as an alternative source of $^{\bullet}NO$ under hypoxic/inflammatory conditions. This is evidenced by *in vivo* studies demonstrating diminution of beneficial outcomes attributable to NO_2^- upon pre- or co-treatment with xanthine oxidoreductase (XOR) inhibitors. However, there is ongoing concern regarding potential off-target effects of currently utilized XOR inhibitors including allo/oxypurinol, febuxostat and Na-tungstate. This is notably true when regarding the potential impact on purine catabolic pathways that result in alteration in levels of adenosine, a potent signaling agent in the vasculature. As such, we attempted to validate results obtained with these pharmacologic agents using our recently developed liver-specific conditional *xdh* knockout model. The *cre/lox* system was used in a C57Blk/6J background to generate *xdh* "*floxed*" ($xdh^{fl/fl}$) mice which were bred with mice expressing *cre* recombinase in hepatocytes. The resultant liver-specific $xdh^{-/-}$ knockout demonstrates 96% diminution of XOR activity in the absence of deleterious phenotypic issues and without altering XOR activity in the lung, heart, skeletal muscle, kidney and adipose tissue. Livers from $xdh^{-/-}$, $xdh^{fl/fl}$ $cre^{-/-}$ littermates and wild-type controls were harvested and homogenates were analyzed for NO_2^- reductase activity using enhanced chemiluminescence. When compared to $xdh^{fl/fl}$ $cre^{-/-}$ littermates and wild-type controls, $xdh^{-/-}$ livers demonstrated 78% less NO_2^- reductase activity. This value is greater than, but similar to livers treated with febuxostat (Uloric®) (76%) or allopurinol (68%). Nitrite reductase activity was reduced to near baseline rates when homogenates treated with CO + febuxostat suggesting that heme-catalyzed reductive mechanisms were responsible for the proportion of $^{\bullet}NO$ formed independent of XOR. Combined, these data serve to validate XOR-catalyzed $^{\bullet}NO$ generation as a novel function for XOR; one that may play a pivotal role in vascular responses to toxicant exposure whereby treatment with nitrite may countervail XOR-mediated ROS generation to produce salutary outcomes.

CYTOTOXICITY OF RESPIRABLE SURFACE-TREATED/UNTREATED CALCITE ROCK DUST PARTICLES IN HUMAN MACROPHAGES

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Calcium carbonate ($CaCO_3$) rock dust (RD) is used in the mining industry to reduce the explosibility of aerosolized coal dust, but, during the dusting procedures, the potential for human exposure occurs, raising health concerns. In addition, to improve the RD aerosolization, several types of anti-caking surface treatments exist. The aim of the study was to evaluate cytotoxicity of 4 respirable RD samples: untreated/treated limestone (L/TL), untreated/treated marble (M/TM), and crystalline silica (SiO_2) as a positive control in a THP-1 human macrophage model. The coating on TM RD was stearate-based, while the coating on TL RD was based on silicone. Free SiO_2 content in the RD samples was less than 1%. Respirable fractions were generated and collected using FSP10 high flow-rate cyclone samplers. After differentiation with PMA (20 ng/ml, 48 h), cells were first exposed for 24 h to 7 different concentrations of RD and SiO_2 ranging from 0 - 0.06 mg/cm^2. At 24 hours, there was significant dose-dependent lactate dehydrogenase, inflammatory cytokines and chemokines release as well as increased cas-1 activity in SiO_2- and TM - exposed cells, but not in other RD. L, TL and M rock dust samples caused some LDH leakage and different cytokine responses, albeit only at highest doses. To test if the increased toxicity of the TM was uptake-related, THP-1 cells were treated with phagocytosis inhibitor Cytochalasin D (CytD) or inhibitor of vacuolar proton pump Bafylomycin A (BafA), followed by exposure to RD or SiO_2 for 6 hours. CytD treatment blocked the uptake and mitigated or significantly decreased cytotoxic effects in all samples. BafA completely prevented cas-1 activation and partially rescued SiO_2- but not TM-exposed cells. The obtained results demonstrated, that out of all RD samples, only stearate-treated particles were able to induce some inflammatory response in THP-1 cells, however it was much less pronounced compared to SiO_2.

DISCOVERY OF A NOVEL ERGOT ALKALOID GLYCOSIDE FROM METABOLOMIC ANALYSIS OF *IPOMOEA* SPECIES

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Ergot alkaloids are fungal tryptophan derived toxins which affect human circulation and neurotransmission. Several *Ipomoea* species and related plants in the morning glory family harbor vertically-transmitted, symbiotic fungi in the genus *Periglandula* that produce ergot alkaloids. Many additional *Ipomoea* species are found to contain ergot alkaloids, indicating symbiosis with uncharacterized *Periglandula* species. We used a metabolomics approach to investigate biomarkers of fungal infection, which could indicate the presence of non-ergot producing cryptic *Periglandula* species. We studied the metabolomes of *Ipomoea tricolor* seeds collected from *Periglandula* sp.-infected plants (P+) or plants that had been cured by treatment with fungicide (P-). Seed extracts were screened for ergot alkaloids by fluorescence HPLC, and total metabolites by mass spectrometry. Previously reported ergot alkaloids were present in high concentrations in P+ seeds, and were not detected in P- seeds. Amino acid concentrations and detected plant stress hormones did not differ significantly between treatments. Analytes that were significantly more abundant in P+ seeds compared to P- seeds were compared to metabolomes from seed extracts of ergot alkaloid-positive and ergot alkaloid-negative seeds of *Ipomoea parasitica* and *Ipomoea pes-caprae,* as well as from nine ergot alkaloid-deficient *Ipomoea* species. Four metabolites tracked the presence of symbiont in this survey. One of these compounds, identified by MS/MS analysis, is a previously uncharacterized form of the pharmaceutically important ergot alkaloid, ergonovine. The data indicate that apart from the accumulation of ergot alkaloids, *Periglandula* species have a minimal impact on the metabolome of seeds of their host plants. Furthermore, we found no evidence of cryptic, non-ergot alkaloid producing *Periglandula* species in seeds of the nine additional *Ipomoea* species analyzed.

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FRACKING SAND DUST ELICITS ROS RESPONSE FROM MURINE MACROPHAGE CELLS

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Hydraulic fracturing is used in the majority of natural gas wells across the United States. Water, sand, and chemicals are delivered at high pressure into drilled wells to fracture the shale and extract natural gas. Fracking sand (comprised mainly of silica) is used to keep these fissures open. In this study, murine macrophage cells (RAW 264.7) were used to investigate cellular responses to the soluble and insoluble components of fracking sand dust (FSD). FSD was washed in PBS two separate times to allow soluble material to be released. The supernatant was collected after 5 days of washing (1st wash), then fresh PBS was added to the FSD to wash for another 5 days and collected (2nd wash). The washed sand was re-suspended in PBS (Washed), and fresh fracking sand was also suspended in PBS (Unwashed). This procedure was repeated with Min-U-Sil (Min) so that comparisons could be made to high purity silica. Cells were treated with final concentrations of 1 mg/ml and 5 mg/ml. After a 24 h exposure with 5 mg/ml of washed and unwashed FSD, the viability of RAW 264.7 cells decreased significantly compared to PBS treated controls, while the 1st and 2nd washes had no significant effect. The washed FSD caused the most LDH release, while the 1st and 2nd wash supernatants and the unwashed FSD had no discernable effect. Acellular production of the hydroxyl radical (·OH), was the highest in unwashed FSD, followed by the 1st wash supernatant. The 1st and 2nd wash FSD supernatants generated large amounts of intracellular ROS, measured using a cell-permeable probe. DNA damage was elevated after 24 h with 5 mg/ml of the soluble and insoluble FSD suspensions. Our results indicate that FSD is cytotoxic to RAW 264.7 cells, as evidenced by decreases in viability and increases in membrane and DNA damage. The washed and unwashed FSD elicit differential responses, as well as the soluble and insoluble portions of FSD. These responses indicate multiple mechanisms of fracking sand toxicity, and warrant further studies into the components of FSD.

A NETWORK APPROACH OF THE SPATIOTEMPORAL PHOSPHOPROTEIN SIGNALING IN A MOUSE MODEL OF GULF WAR ILLNESS

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The 1991 Persian Gulf War (GW) included nearly 700,000 troops from the United States and other partnering countries. Today, approximately 30% of the veterans who served in the GW suffer from a multitude of medically unexplained chronic symptoms referred to as Gulf War Illness (GWI). GW veterans were reportedly exposed to multiple acetylcholinesterase (AChE) inhibitors and high physiological stress that may have served as initiating events for the pathobiology currently associated with GWI (e.g. chronic neuroinflammation). In response to these insults, which included organophosphates, an investigation into the early inflammatory responses that occur within the brain of rodents post-exposure to a stress hormone and a sarin mimic was undertaken. Specifically, adult male C57BL/6J mice were exposed to corticosterone (Cort; 200 µg/mL) in the drinking water for 7 days, and on the 8th day, were given a single intraperitoneal injection of diisopropyl fluorophosphate (DFP, a sarin surrogate; 4.0 mg/kg). Mice were euthanized 30 minutes, 2 hours, and 24 hours post-exposure via focused microwave radiation. To fully understand the effects of DFP and DFP+Cort on the brain, post-translationally modified protein targets were measured using multiplex ELISA. The early post-translational phosphorylation responses were measured (e.g. syk, p90RSK, CREB, etc.); these are thought to regulate stress pathways and influence neuroinflammation. To analyze this large dataset, we used network analytics, and specifically node centrality to describe how central one node is, relative to all other nodes (e.g. post-translational modifications) in the system. To optimize the analysis of the data sets, centrality parameters corresponding to radiality were used to assess the response of the phosphoprotein targets that were highly impactful relative to all other targets in the system. This approach holds the potential to discern a portion of the etiology of GWI based on the elucidation of the relevant spatiotemporal phosphorylation responses.

CRUDE OIL VAPOR EFFECTS UPON AIRWAY EPITHELIAL ION TRANSPORT AND LUNG FUNCTION IN THE RAT

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Crude oil vapor (COV) is a mixture of hydrocarbon vapors and volatile organic compounds (VOCs). Workers in the oil and gas industry are potentially exposed to COV while conducting routine tasks such as manual sampling, gauging and filling crude oil storage tanks from oil tanker trucks. The effects of COV inhalation exposure on the pulmonary system are unknown. Previously, we found there were no significant changes in pulmonary function or airway epithelial ion transport after acute inhalation exposure to COV (300 ppm total VOCs, 6 h/d, 1 d). In the current study, the effects of a sub-chronic inhalation exposure of COV on lung function and epithelial ion transport were investigated. Rats in whole body chambers were exposed to 300 ppm total VOCs for 28 d. Experimental endpoints were measured at 18 h, 28 and 90 d post-exposure. Total VOCs, benzene, toluene, ethylbenzene, and xylene concentrations were monitored and regulated during exposures to maintain concentration consistency. Transepithelial potential difference (V_t), transepithelial resistance (R_t), and short-circuit current (I_{SC}) were measured in tracheas mounted in Ussing chambers and treated with the ion transport inhibitors amiloride (Na^+ channel blocker; apical), 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB; Cl^- channel blocker; apical), and ouabain (Na^+,K^+-pump blocker; basolateral). Compared to air-breathing controls, the I_{SC} response to NPPB was increased significantly at 28 d post-exposure, indicating an increase in Cl^- transport in the airway epithelium. There were no changes in V_t or R_t at 18 h or 28 d post-exposure. Lung resistance (R_L), dynamic compliance (C_{dyn}) and reactivity to inhaled methacholine (MCh) were measured in anesthetized rats. COV significantly increased basal R_L compared to air-breathing controls at 90 d post-exposure. There was no effect of COV on basal C_{dyn} or reactivity to inhaled MCh at any time point. Our results indicate that sub-chronic exposure to COV changes airway ion transport and pulmonary function.

EVALUATION OF pH-TARGETING FLUORESCENT PROBES FOR MAPPING INFLAMMATORY RESPONSE

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Elevated extracellular acidity due to increased glycolytic flux is a hallmark of many pathological states. Exploiting acidosis to develop probes that monitor and track inflammation in real time could be a viable approach to investigate a spectrum of toxicologically-relevant endpoints, from bioenergetic measurements to tumorigenesis. pH-Low-Insertion-Peptide (pHLIP) is a pH-sensitive peptide that is capable of cellular transmembrane insertion through a helix formation mechanism that is reliant on the protonation of aspartic acid residues under localized acidic conditions. Upon insertion, the C-terminus translocates inside the cell while the N-terminus remains extracellular. By tethering a fluorophore to the N-terminus, researchers may visualize inflammatory response, following xenobiotic exposure, in real time, which provides an orthogonal view to complement the findings of biomarker profiling *in vivo. In vitro* studies using pHLIP-1 allow for the targeting and sorting of metabolically compromised cells for further study. Our laboratory has been optimizing pHLIP variants coupled to fluorescein isothiocyanate (FITC) to demonstrate successful targeting of L6 rat skeletal myoblasts post-exposure to mitochondrial electron transport chain inhibitors. Our techniques have achieved 100% localization to acidic cells in under one minute. Furthermore, our *in vitro* work has investigated the binding constants associated with pHLIP to pinpoint optimal concentrations for further *in vivo* work, as well as establishing a dose-response relationship between amount of pHLIP and extent of cellular injury.

THREE DIMENSIONAL (3-D) PRINTER EMISSION-INDUCED CELL TOXICITY

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Three-dimensional (3-D) printing with polymers is known to emit billions of ultrafine particles and volatile organic compounds (VOCs) that are formed by thermal degradation of the filament. The potential toxicity of these emissions is unknown. Emissions were generated using a commercially available 3-D printer while operating with acrylonitrile butadiene styrene (ABS) or polycarbonate (PC) filaments. The samples were collected in serum-free cell culture media and characterized to determine the mean hydrodynamic particle size (nm), particle concentration, and VOCs content. Human small airway epithelial cells (SAEC) were exposed to printer emissions to investigate cellular viability, membrane damage, morphological changes, total antioxidant capacity, glutathione peroxidase, and reactive oxygen species levels at 24 hours post exposure. The mean sizes of background, PC and ABS-emitted particles in culture media were 210 ± 13, 201 ± 8, and 198 ± 10 nm, respectively. The particles concentration of the background, PC and ABS emissions were 0.23×10^7, 3.47×10^7, and 1.51×10^7 particles/ml, respectively. Bisphenol A and styrene were the predominant VOCs collected in the media for the PC and ABS emissions, respectively. No VOCs were detected in background sample. Both PC and ABS emissions significantly decreased SAEC cellular viability and increased cell membrane damage. Transmission electron microscope images indicate that particles were engulfed by SAEC. Only PC caused alterations in antioxidant and pro-oxidant balance. Our data indicate that the emissions generated by PC and ABS filaments induce a toxic response in SAEC.

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POTENTIAL PROTECTIVE ROLE OF NSIO$_2$-COATING IN IRON OXIDE NANOPARTICLE-INDUCED CELLULAR TRANSFORMATION OF HUMAN BRONCHIAL EPITHELIAL CELLS

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Iron oxide nanoparticles (IONP) are emerging as unique components of drug delivery systems, imaging techniques, environmental catalysts, and more. Workers in IONP manufacturing facilities are known to be exposed to low doses of these particles over long periods of time. However, very few studies have assessed potential adverse outcomes following this type of occupationally relevant exposure. Our previous research suggests that IONP will induce a neoplastic-like cellular transformation in primary small airway epithelial cells, likely related to particle dissolution, release of free iron ions, and disruption of iron homeostasis. Other studies suggest that an amorphous silica coating may reduce particle dissolution, thereby reducing subsequent adverse outcomes. We hypothesized that an amorphous silica coating (SiO_2-nFe_2O_3) would reduce iron homeostasis disruption and oxidative stress induced by an uncoated but otherwise identical particle (nFe_2O_3), and would therefore reduce nFe_2O_3-induced neoplastic-like cellular transformation. To test this hypothesis, we used an occupationally relevant low dose/long term *in vitro* exposure using a normal human bronchial epithelial cell line (Beas2B). The cells were continuously treated at 0.6 µg/cm^2 to SiO_2-nFe_2O_3 or nFe_2O_3 for six months, and were evaluated for iron homeostasis disruption, oxidative stress, and neoplastic-like cellular transformation throughout. Our results show an nFe_2O_3 induced cellular transformation beginning at about four months post exposure, as indicated by changes in cellular proliferation and attachment independent colony formation. These cells also show significantly elevated intracellular iron and ROS production at the same time point. These outcomes are not seen with SiO_2-nFe_2O_3 exposure. Overall, our results suggest that sub-chronic exposure to nFe_2O_3 will induce cellular iron homeostasis disruption, oxidative stress, and cellular transformation, which could be protected with an amorphous silica coating. This data provides novel information of low dose/long term nFe_2O_3 exposure induced adverse outcomes, and the utility of a surface coating as a promising component for safe-by-design hazard reduction strategy.

BIOACTIVITY AND TOXICOLOGICAL EVALUATION OF A MULTI-ASPECT-RATIO, MULTI-WALLED CARBON-NANOTUBE MIXTURE.

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Multi-walled carbon nanotube (MWCNT) as a reinforcement to traditional constructional material matrices is revolutionizing the industry. These novel nanocomposites have vastly superior mechanical and structural properties compared to traditional materials. In order to be cost effective and to improve the distribution in the composite matrix, MWCNTs with a multi-aspect ratio are being utilized. The incorporated MWCNTs have a distribution from a few nanometers up to 20 micrometers in length and a diameter that changes correspondingly. Nanotoxicological research over the past decade has identified pulmonary health effects and the associated molecular mechanisms associated with exposure to uniform tubes; however, not much is known when exposure is associated with multi-aspect ratio tubes. The toxicity profile of two multi-aspect ratio MWCNTs (MWCNT1 & MWCNT2) was compared to a more uniform MWCNT (MWCNT-7) with a larger diameter (~50 nm) , and carbon black (CB, printex-90). The toxicity potential was assessed by screening the various particulates in the human monocytic cell line (THP-1), both wild-type and NLRP3-inflammasome-deficient cells, murine alveolar macrophages (RAW 264.7), and primary human lung fibroblast cells (PHF) over a wide dose range (0 – 120 µg/ml). Lactate dehydrogenase (LDH) activity, a marker of cytotoxicity was assessed in the aforementioned cell types. Cell proliferation and collagen 1 production in PHF, exposed to the test particulate (0 - 2 µg/cm^2), did not discriminate the multi-aspect ratio particulate. There was an approximately 150-fold change in IL-1β secreted from THP-1 WT vs. NLRP3-deficient cells; furthermore, all of the tested particulates had a dose-dependent increase in IL-1β. The change in NLRP3 dependent IL-1β ranked in the order MWCNT2, MWCNT-7, CB and MWCNT1. There was no correlation between the acellular oxidative stress potential measured using ferric-reducing ability of serum assay and *in vitro* oxidative stress induced in RAW 264.7 cells. In conclusion, evaluation of general cytotoxicity, inflammation, and fibrogenic potential showed no discrimination of the multi-aspect ratio particulate compared to uniformed tubes and the toxicity profile was more dependent on the other physicochemical characteristics of the nanotubes.

FARNESOID X RECEPTOR AGONSITS REDUCE CYTOKINE-INDUCED INFLAMMATORY RESPONSE OF IMMORTALIZED AND PRIMARY MOUSE ASTROCYTES

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Neuroinflammation is a significant contributor to the initiation and progression of many neurodegenerative diseases including Parkinson's disease. Pro-inflammatory cytokines are released by microglia during a neuroinflammatory response, which activates astrocytes through the NF-κB pathway. Once activated, astrocytes release reactive molecules, including nitric oxide, which can damage neighboring neurons. The farnesoid X receptor (FXR) is a nuclear receptor known for regulating bile acid metabolism and hepatic inflammation. We have previously shown that $FXR^{-/-}$ mice exhibit reactive neuroinflammation and dopamine neuron loss, suggesting that FXR may also regulate neuroinflammation. Here we demonstrate that in the brain FXR is primarily expressed in astrocytes. To assess the role of FXR in neuroinflammation, we treated immortalized and primary mouse astrocytes (IMA and PMA) with a cytokine mixture (CM) consisting of TNF-α, IL-1β, and IFN-y in the presence or absence of 0.1-10 μM of synthetic FXR agonists GW4064 (GW) or WAY-362450 (WAY). We then assessed levels of media nitrite, an index of iNOS induction, and used qPCR to assess *iNos* and *Shp* gene expression, which are indicators of pro-inflammatory response and FXR stimulation respectively. The data indicate treatment of IMA and PMA with GW or WAY increased *Shp* gene expression in both. Treatment of IMA or PMA with CM significantly increased media nitrite and *iNos* gene expression when compared to control. Co-treatment of IMA or PMA with CM and 10 μM GW or WAY significantly attenuated media nitrite and *iNos* gene expression. These data demonstrate that FXR plays a role in the cytokine-mediated inflammatory response of astrocytes, and could represent a novel therapeutic target for reducing neuroinflammation linked to neurodegenerative disease.

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ACUTE EFFECTS OF E-CIGARETTE VAPOR EXPOSURE ON PERIPHERAL VASCULATURE

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Introduction: Recent studies have shown impairment of the vascular endothelium upon exposure to electronic cigarette (E-cig) vapor. Little is currently known about the duration or severity of the effects of E-cig vapor with or without nicotine on the peripheral arterioles. We hypothesize that E-cigs will adversely effect vessel tone and dilatory capacity in the gluteus maximus arterioles.
Methods: Wild type C57BL/6J mice were anesthetized with intraperitoneal inactin (Sigma-Aldrich) and a tracheal tube inserted. A portion of the gluteus maximus muscle was surgically externalized and incubated in a physiological saline bath at body temperature. First order arterioles were imaged at baseline for 5 mins, followed by exposure to 10^{-3} M acetylcholine (ACh) for 5 minutes. Baseline and dilation to ACh were used as pre-exposure controls for repeated measures post exposure. Thereafter, mice were exposed to 5 mins of French Vanilla flavored E-cig vapor with (18 mg/ml) or without (0 mg/ml) nicotine (50:50 VG:PG). Images were taken immediately after exposure and at 15 min intervals for up to 2 hours. Images of vessel diameter at each time point were measured in triplicate using a calibrated NIH Image J software.
Results: Baseline arteriole diameter were not different in mice exposed to E-cig vapor with and without nicotine (23.0 and 22.7 um, respectively). After exposure to E-cig vapor, arteriole diameters decreased by an average of 4.3 um and 5.9 um in nicotine vs no nicotine vape groups, respectively ($p<0.05$). This resulted in 19% and 26% reduction in arteriole diameters in nicotine vs. no-nicotine vapor exposed mice, respectively ($p<0.05$).
Conclusions: These are preliminary data from an on-going study reporting the acute temporal in vivo response of peripheral arteriole diameters in mice exposed to E-cig vapor with and without nicotine. Our findings suggest that E-cig vapor without nicotine exhibits a similar vasoconstriction effect at one-hour post exposure compared to vapor with nicotine.

Subchronic *in vitro-in vivo* models for the assessment of iron oxide nanoparticle pulmonary toxicity

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Potential human exposure to nano-scaled respirable particles (NPs) has become a major concern with increasing evidence showing that NP pulmonary exposure results in particle deposition in deep lung tissues and causes pathological changes. Such adverse health effects have not been well assessed due to several technical challenges and knowledge gaps, including 1) limitations of specific *in vitro* models to evaluate the vast variety of NPs, while it is impossible to assess the toxicities of countless NPs using animal models; 2) inconsistency of *in vitro* delivered doses from the administration doses may limit conclusive findings; and 3) changes in physicochemical properties and bioactivities of NPs under submerged conditions which do not represent *in vivo* exposure conditions of inhaled "dry" NPs. To address the knowledge gap and technical limitations, we have developed multiple *in vitro* models to assess the biological and toxicological activities of well characterized NPs, including iron oxide (nFe_2O_3), with the identification of target lung cells at the site of particle accumulation *in vivo*. Based on established *in vivo* doses that induce significant pulmonary disorders in animal models, physiologically relevant *in vitro* doses (0.02 - 0.2 $\mu g/cm^2$) of nFe_2O_3 were used to evaluate the toxic effects of NPs on human lung cells under a long-term exposure condition (up to 6.5 months). Our study data showed that nFe_2O_3 was able to induce dose- and time-dependent cytotoxicity, DNA damage, cell proliferation, anchorage-independent growth of human bronchial epithelial cell (Beas-2B). Furthermore, such *in vitro* models allow us to examine underlying mechanisms, for example, to identify key signaling pathways and mediators involved in nFe_2O_3-induced pathological processes (e.g. cytokines, growth factors, and reactive oxygen species), which may serve as predictive biomarkers for *in vivo* pathological responses. We also investigated a 3D air-liquid interface model to mimic pulmonary NP exposure condition and to test its potential utility as a predictive *in vitro* model in combination with animal study using the same aerosolized NPs. The described integrated *in vivo-in vitro* approach will support the utility of *in vitro* models as rapid screening and predictive tools for risk assessment of nanomaterials.

CARBON NANOTUBES INDUCE MATRIX REMODELING AND CONTRACTION BY STIMULATING MYOFIBROBLAST TRANSFORMATION IN A THREE-DIMENSIONAL CULTURE OF HUMAN PULMONARY FIBROBLASTS

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Background: Pulmonary fibrosis is a common but poorly understood pathologic condition in many lung diseases. Carbon nanotubes (CNTs) are nanomaterials used in a wide range of applications, and can induce pulmonary fibrosis in animals, a cause for concern for exposed workers and consumers. Given the large number of CNTs available, and the seemingly infinite number of ways these particles can be modified, in vitro models that can be used to quickly and efficiently investigate the relative fibrogenicity of CNTs are much needed. Here we analyzed the fibrogenic potentials of six CNTs using two and three-dimensional (2D and 3D, respectively) in vitro models.

Methods: WI38-VA13 human pulmonary fibroblasts were treated with CNTs of differing physical properties, silica, carbon black (CB), TGF-β1, or vehicle control. Exposed cells were examined for myofibroblast differentiation, matrix remodeling, and matrix contraction.

Results: While all tested CNTs induced myofibroblast differentiation as shown by α-SMA expression in both 2D and 3D models, the 3D collagen gel model allowed for the examination of the morphology of activated myofibroblasts, as well as their interactions with CNTs and other cells in the matrix. Moreover, the 3D culture enabled the observation of myofibroblast clustering, collagen deposition and rearrangement, and matrix contraction in response to CNT exposure, processes critical for fibrosis development in vivo. At 1 µg/ml, MWCNTs elicited higher induction of myofibroblast differentiation and matrix remodeling than SWCNTs. Among the MWCNTs, those with highest and lowest aspect ratios produced the largest effects.

Conclusion: The 3D collagen-based model of fibrosis can be used to analyze the induction of myofibroblast activation by CNTs and other particulate inducers effectively and quantitatively to determine their relative fibrogenic potentials in vitro.

IN VIVO TOXICOLOGICAL ASSESSMENT OF SANDING DUST GENERATED FROM MICRONIZED COPPER AZOLE PRESSURIZED TREATED WOOD

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Micronized copper azole (μCAC) is a third generation pressurized lumber treatment to prevent weathering and fungal infiltrations. In this study, the *in vivo* toxicity of sanding dust generated from μCAC was compared to that of copper azole treated (CAC) or untreated yellow pine (UYP) wood to determine if the micronized copper was more bioactive than elemental copper. Particle matter ($PM_{2.5}$) was collected from sanding dust of each type of lumber and analyzed for metal content, using inductively coupled plasma mass spectrometry. μCAC had a higher concentration of copper and generated smaller particles in comparison to CAC and UYP. Mice were exposed to three doses (28, 140, 280 μg) of UYP, μCAC, or CAC, using pharyngeal aspiration. Lung damage and inflammation were examined 1 and 7 day post exposure, using bronchoalveolar lavage fluid (BALF) by measuring lactate dehydrogenase (LDH) activity and polymorphonuclear cells (PMNs). Results showed that LDH activity was significantly increased at 1 day post exposure for 280 μg of μCAC and CAC compared to UYP. μCAC and CAC caused a dose-dependent increase in PMNs. There were also increases in pro-inflammatory cytokines with the BALF from the μCAC and CAC exposed groups at 1 day post-exposure. There were no significant changes seen at 7 day post-exposure. Exposure to all materials resulted in acute inflammation with infiltration of neutrophils and macrophages. The pulmonary response was more severe in the μCAC and the CAC groups. Furthermore, μCAC caused a more severe inflammatory response than CAC at 1 day post exposure. At 84 day post exposure, there were no significant changes observed in any exposure group compared to saline control. These data suggest that μCAC and CAC are both more bioactive than UYP; and, moreover, μCAC is slightly more toxic in comparison to CAC.

EVALUATION OF THE TOXICITY OF MILD STEEL AND STAINLESS STEEL WELDING FUME PARTICLES ON HUMAN PLACENTAL CELLS

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According to the U.S. Department of Labor Women's Bureau, the number of female welders in the United States has increased over the past decade. Since men have traditionally comprised the bulk of the construction workforce, the majority of research performed on welding fume exposure in relation to reproductive toxicology has mainly focused on the adverse effects of exposure on sperm. With the trend of more women entering the welding workforce, it is imperative that the adverse effects of welding fume exposure on female reproductive organs also be explored. In this study, human placental trophoblast cells (HTR-8/SVneo) obtained during the first trimester of pregnancy were used to better understand the mechanisms of toxicity associated with stainless steel (GMAW-SS, SMAW-SS) and mild steel (GMAW-MS) exposures. The potential adverse effects of welding fumes on the placenta can occur by exposure to the solubilized metals in the maternal bloodstream, or by the presence of non-soluble welding particles which can cross the placental barrier if < 100 nm in size. Fumes generated from MS are mainly comprised of iron and manganese, while SS welding fumes also contain hexavalent chromium [Cr(VI)] and nickel. Most recently, the International Agency for Research on Cancer, classified welding fumes as a Group 1 carcinogen. We hypothesized that the presence of these metals would play a role in the pro-inflammatory responses and cytotoxicities observed. For all experiments, cells were either treated with welding fumes for 4 h or 24 h. The low dose used was 10 µg/ml, while the high dose was 100 µg/ml. Compared to GMAW-MS, GMAW-SS caused a significantly greater decrease in cellular viability, as measured via the WST-1 assay. However, SMAW-SS, which contained the highest levels of Cr(VI) of all the welding fumes (38000 ppm), caused 100% cell death at the high dose at 24 h. Measured via electron paramagnetic resonance (EPR), SMAW-SS also produced a greater amount of hydroxyl radicals as compared to GMAW-MS and GMAW-SS, whereas all three welding fumes generated significant amounts of intracellular ROS over time. Using ELISA, production of the pro-inflammatory mediator IL-8 was also measured. IL-8 is constitutively produced by placental cells, and exerts chemotactic and activating activity on neutrophils. Upon exposure to all three welding fumes, IL-8 levels were significantly increased in HTR-8/SVneo cells when compared to negative controls at 24 h. With such little data available on the effects of welding fume exposure on the female reproductive system, our results provide valuable insights on this understudied potential health issue.

DERMAL TRICLOSAN EXPOSURE INDUCES MITOCHONDRIAL MODIFICATION, AUGMENTING IMMUNE FUNCTION *IN VIVO*

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Triclosan has had widespread use in the general population as an antimicrobial agent. Recently, concerns of triclosan's toxicological effects has attracted attention of the scientific community, regulatory agencies and the general public, leading to triclosan being phased out of consumer products. However, triclosan is still used occupationally in clinical applications, making its use a remaining area of concern. Urinary levels of triclosan show a positive association with the diagnosis of allergies, hay fever, and sensitization to aeroallergens and foods. In a mouse asthma model, work by our group has shown that dermal exposure to triclosan augmented the allergic response to a known allergen through a TSLP-mediated pathway. We have also shown that dermal triclosan exposure augments the innate immune response via the S100A8/A9-TLR4 pathway. Previous *in vitro* studies show that triclosan alters immune mast cell function through effects on mitochondrial morphology, calcium levels, translocation, and membrane potential. Mitochondria have recently been shown to be involved in T cell immune function through effects on differentiation and activation. The following studies were conducted to further evaluate the connection between mitochondria and immune function following dermal triclosan exposure. BALB/c mice were exposed dermally on the ears to concentrations of triclosan ranging from 0.75-3% (0.375-1.5mg/mouse/day) for up to 7 consecutive days. Expression of mitochondrial genes in the ears and draining lymph nodes following exposure was analyzed using quantitative polymerase chain reaction. Robust dose-responsive decreases in *Mfn1* and *2*, *Opa1*, *Rhot1 (Miro1)*, *Taz*, *Slc25a27*, and *Slc25a1* were observed in the ears. In the lymph node draining the exposure site, dose responsive increases in *Slc25a27* and decreases in *Slc25a1* and *Rhot1* were observed. In genes associated with mitochondrial involvement, a decrease in *NF-κB*, *IL-18*, and *IL-2* and a robust increase in *IL-1β* was observed in the ear following dermal exposure to triclosan. These results suggest that triclosan may augment allergic responses through modulation of mitochondrial function, and may point to novel ways to assess chemical allergic potential.

USING LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC-MS) TO ASSESS THE EFFECT OF AGE, DIET, AND RAT STRAIN ON THE GLOBAL METABOLOME

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The exposome encompasses the entire environmental exposures of an individual during a lifetime. These exposures include diet, lifestyle, environmental toxins, and workplace exposures. The interactions of combined exposures can lead to exacerbation of disease. The goal of this study was to use liquid chromatography mass spectrometry (LC-MS) to assess metabolic changes in three distinct animal strains based on two different diets. Sprague-Dawley (SD), Fischer 344 (F344), and Brown-Norway (BN) male rats were maintained on a high fat, Western (HF), or regular diet for 24 weeks. Serum was collected at baseline, 4, 12, and 24 weeks to assess global small molecule metabolite changes. A cold methanol buffer was used to pellet proteins and extract polar metabolites. Metabolite extracts were then analyzed using high resolution accurate mass (HRAM) mass spectrometry coupled to ultra-high performance liquid chromatography. The results of the global metabolomics revealed significant changes based on both age and diet within all three strains. Principal component analysis revealed that the influence of age caused a greater variation in the significantly changing metabolites ($p \leq 0.05$) than that of diet for the BN and F344 strains, while the SD strain showed a large influence from diet at the 4 week time point. As expected, metabolites involved in lipid metabolism and bile acid formation were upregulated in the animals maintained on a HF diet compared to the regular diet. There were also significant changes observed in acetyl-coA concentrations between the two diets at all of the time points for all strains. A total of 12 different coA species were quantified from all experimental groups. Future studies will combine occupational exposures with age, diet, and animal strain differences in further assessment of the exposome.

COMPARISON OF THE TOXICOLOGICAL EFFECTS OF MULTI-WALLED CARBON NANOTUBES AND NITROGEN-DOPED MULTI-WALLED CARBON NANOTUBES ON RAT LUNG FUNCTION

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The toxicological effects of multi-walled carbon nanotubes (MWCNT) have been widely investigated. Outcomes can range from initiating pulmonary inflammation and fibrosis to cardiovascular and central nervous system effects in animal models. Previous studies in our laboratory have shown that inhalation of MWCNT (Mitsui-7) results in airway hyperreactivity to methacholine (MCh) in rats. Nitrogen-doped multi-walled carbon nanotubes (N_2-MWCNT) are MWCNT that have been functionalized to incorporate nitrogen. They are utilized in applications such as lithium batteries and as matrix fillers in composite materials. N_2-MWCNT are shorter and more brittle than their MWCNT counterparts, which may reduce toxicity. The purpose of this study was to determine if exposure to N_2-MWCNT results in alterations in lung function that are comparable to those observed after MWCNT exposure. Sprague-Dawley rats were administered MWCNT or N_2-MWCNT (25, 50, or 250 µg) in dispersion medium by intratracheal instillation. Lung resistance (R_L), dynamic compliance (C_{dyn}), and reactivity to MCh aerosol were examined at 1 and 7 days post-exposure. Animals exhibited increased reactivity to MCh at 1 day after treatment with 25 and 250 µg MWCNT. Additionally, baseline R_L was decreased at 7 days after treatment with 50 µg MWCNT without a corresponding change in reactivity to MCh. The only alteration observed in N_2-MWCNT exposed animals was a decrease in baseline R_L at 7 days after 250 µg N_2-MWCNT exposure, with no corresponding change in reactivity to MCh. These results suggest that N_2-MWCNT are less toxic than MWCNT with respect to lung function.

CHARACTERIZATION OF FUNCTIONAL AND MOLECULAR ENDPOINTS OF POTENTIAL ADVERSE HEALTH EFFECTS ASSOCIATED WITH AGE, DIET, AND OCCUPATIONAL EXPOSURE IN AN ANIMAL MODEL

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The exposome is the measure of all exposures of an individual in a lifetime from conception to death and how those exposures affect health. An individual's exposome is highly variable and dynamic throughout their lifetime. The goal was to design an exposure paradigm that would address multiple exposome components, including lifestyle (e.g., diet), age, and occupational exposure (welding fume; WF) in a controlled animal model. Functional and molecular endpoints predictive of adverse health effects in recovered biological fluids of exposed animals that are translatable to human populations were examined. Male Fischer 344 rats were maintained on a high fat western (HF) or regular (REG) diet for 24 wk. At wk 7 during diet maintenance, groups of rats were exposed by inhalation of stainless steel WF (20 mg/m^3 x 3 hr/d x 4 d/wk x 5 wk) or filtered air (control) until wk 12 at which time some animals were euthanized. A separate set of rats were allowed to recover from WF exposure until the end of the 24 wk period. Whole blood and bronchoalveolar lavage fluid were collected at 7 wk (baseline before WF exposure), 12, and 24 wk to assess blood cell differential and to recover serum, peripheral blood mononuclear cells (PBMCs), and lung phagocytes for epigenetic analysis and immune response. Significantly elevated % change in body weight and serum triglycerides were observed in groups maintained on the HF diet. At nearly all time points, phagocytosis of bacteria by recovered phagocytes and PBMC telomere length were significantly decreased in the REG+WF, HF+air, and HF+WF groups compared to the REG+air group. A significant decrease also was observed in telomere length over the 24 wk regimen in all groups. In summary, age, diet, and occupational exposure (WF inhalation), important exposome components, altered immune response and epigenetic endpoints in rats. An animal model may be advantageous for studying the exposome because of the ability to control all external exposures and to measure potential adverse health outcomes of each animal over its entire lifespan and to link a specific internal biological response/endpoint with a specific exposure.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the NIOSH.

ALTERATIONS IN THE EXPRESSION OF SHELTERIN COMPLEX GENES IN CRYSTALLINE SILICA EXPOSED RAT LUNGS

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Occupational exposure to silica can result in advanced pulmonary fibrosis and lung carcinoma through several complex mechanisms. Therefore, it is imperative to identify the key biomarkers of silica-induced pulmonary toxicity for the intervention of lung pathologies. Telomeres (the nucleoprotein structures with repetitive (TTAGGG) sequences at the end of chromosomes) are a molecular “clock of life” and alterations are associated with several chronic diseases. Shelterin complex: protection of telomerase1 (POT1), telomeric repeat-binding factor1 (TRF1), telomeric repeat-binding factor2 (TRF2), TRF1-interacting nuclear factor2 (Tin2), TRF2-interacting telomeric protein (Rap1), and POT1 and Tin2-organizing protein (TPP1) play an important role in maintaining telomere length and integrity and any alteration in telomeres activate DNA damage machinery resulting in telomere attrition. The goal of this study was to assess the effect of crystalline silica exposure on the regulation of shelterin complex genes in an animal model. Male Fisher 344 rats were exposed by inhalation to Min-U-Sil 5 silica for 3, 6, and 12 weeks at a concentration of 15 mg/m^3 for 6 hours/day for 5 consecutive days/week. After the final day of exposure the right lung was homogenized, total RNA was isolated and reverse transcribed to obtain cDNA, and expression of shelterin complex genes was assessed. At all-time points after exposure, mRNA expression of POT1, TRF1, TRF2, Tin2, Rap1, and TPP1 were significantly decreased ($p<0.05$) in the silica-exposed animals compared to air controls, and the decrease observed were exposure time dependent. POT1 and TPP1 which mediates telomerase-dependent telomere extension were significantly decreased in exposed animals. In conclusion, our results suggested that silica inhalation promoted shelterin complex instability. This study indicated that measurement of expressions of shelterin genes involved in telomere regulation may serve as a potential biomarker for silica-induced pathology including carcinogenesis. In addition, changes in shelterin complex could potentially promote telomere end-to-end fusions and cancer formation.

ADVERSE OUTCOME PATHWAY ASSESSMENT OF ORGANOMODIFIED NANOCLAY PULMONARY TOXICITY USING *IN VITRO* HIGH CONTENT SCREENING: CORRELATIONS WITH *IN VIVO* EFFECT

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Nanoclay-enabled composite technology continues to expand based on incorporation of organomodified nanoclay (ONC), montmorillonite coated with different quaternary ammonium compounds, within polymer matrices. Given the forecasts for airborne occupational exposures to occur, little is known about pulmonary health risks along the ONC material life cycle. Our recent *in vivo* exposure study indicated that both pre- and post-incinerated ONCs caused low grade, persistent inflammation with a pro-fibrotic signaling response at day 28 post-exposure, with unknown modes of action. This study hypothesized that both pre- and post-incinerated ONC exposure elicits differential modes of action on pulmonary cells compared to uncoated nanoclay, and that relevant human *in vitro* models correlate with *in vivo* effects. To assess ONC material life cycle pulmonary toxicity, differentiated human monocytes (THP-1), small airway epithelial (SAEC), and primary lung fibroblasts (LF) were evaluated using multiplex fluorescent high content screening for dose-dependent (0-20 μg/cm^2) pulmonary adverse outcome pathways (AOPs). Pre-incinerated uncoated nanoclay (UC) exposure resulted in mild THP-1 macrophage Cathespin B release, LDH release, and apoptosis while ONC (>2 μg/cm^2) caused a robust, dose-dependent necrosis, with little evidence for Caspase 1 activation. Incinerated nanoclays (>2 μg/cm^2) elicited Caspase 1 and 3 activation. UC exposure to SAECs triggered mitochondrial depolarization while CC showed no effect. UC and CC exposure instigated increased LF proliferation, collagen I, pro-collagen III, and fibronectin production, which correlated to similar elevated ECM protein expression in day 28 nanoclay-exposed mouse lung tissue. Incinerated CC (I-CC; 20 μg/cm^2) showed cytotoxicity to all cells while I-UC showed little effect on cellular AOPs. In summary, incineration status and presence of organic coating influences the potential mode of action of the pulmonary response. Specifically, the ONC coating protected against a silica-induced inflammatory response, but induced macrophage plasma and phagosome membrane damage, SAEC membrane damage, and robust stimulation of *in vitro*/*in vivo* LF reticular fiber and collagen production.

Disclaimer: The views expressed in this abstract are those of the authors and do not represent the position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.

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OSTEOPONTIN ENHANCES MULTI-WALLED CARBON NANOTUBE-INDUCED LUNG FIBROSIS

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Carbon nanotubes (CNTs) have been used in a variety of applications owing to their unique physicochemical properties and functions. However, studies have revealed that some CNTs potently induce lung fibrosis in experimental animals, leading to concerns over the potential adverse impacts of CNTs on human health. The fibrosing lesions induced by multi-walled carbon nanotubes (MWCNTs) show a high similarity to pneumoconiosis and idiopathic pulmonary fibrosis (IPF) in humans. Here we analyzed the role of osteopontin (OPN), a cytokine and ECM protein that regulates inflammation, tissue remodeling, and cancer metastasis, in the development of MWCNT-triggered lung fibrosis. OPN was highly and persistently induced by MWCNTs in both the acute and chronic responses in mouse lung tissues and the bronchoalveolar lavage (BAL) fluid. Comparison between WT and OPN knockout (KO) mice revealed that OPN enhanced MWCNT-induced lung fibrosis through promoting the formation of fibrotic foci and increasing the production of matrix proteins in the lungs. At the cellular and molecular levels, OPN promoted TGF-β1 expression and activation, Smad-dependent TGF-β1 signaling activation, fibroblast accumulation, myofibroblast differentiation, and ECM production and deposition in MWCNT-exposed lungs. By using TGF-β1 neutralizing antibodies and a type I TGF-β receptor inhibitor, we demonstrate that OPN enhanced MWCNT-induced fibrotic response through activating TGF-β1 signaling and elevating ECM production in fibroblastic cells. Together, these findings reveal a pro-fibrotic activity of OPN in lung fibroblastic cells exposed to MWCNTs, through which OPN critically aggravates the pulmonary fibrotic response to MWCNT exposure. This study provides new insights into the mechanistic understanding of MWCNT-induced lung fibrosis development, and suggests OPN as a potential biomarker for MWCNT-induced health impact.

INTERACTION OF RESPIRABLE FRACKING SAND DUST (FSD) WITH PULMONARY TISSUES IN VIVO AND IN VITRO

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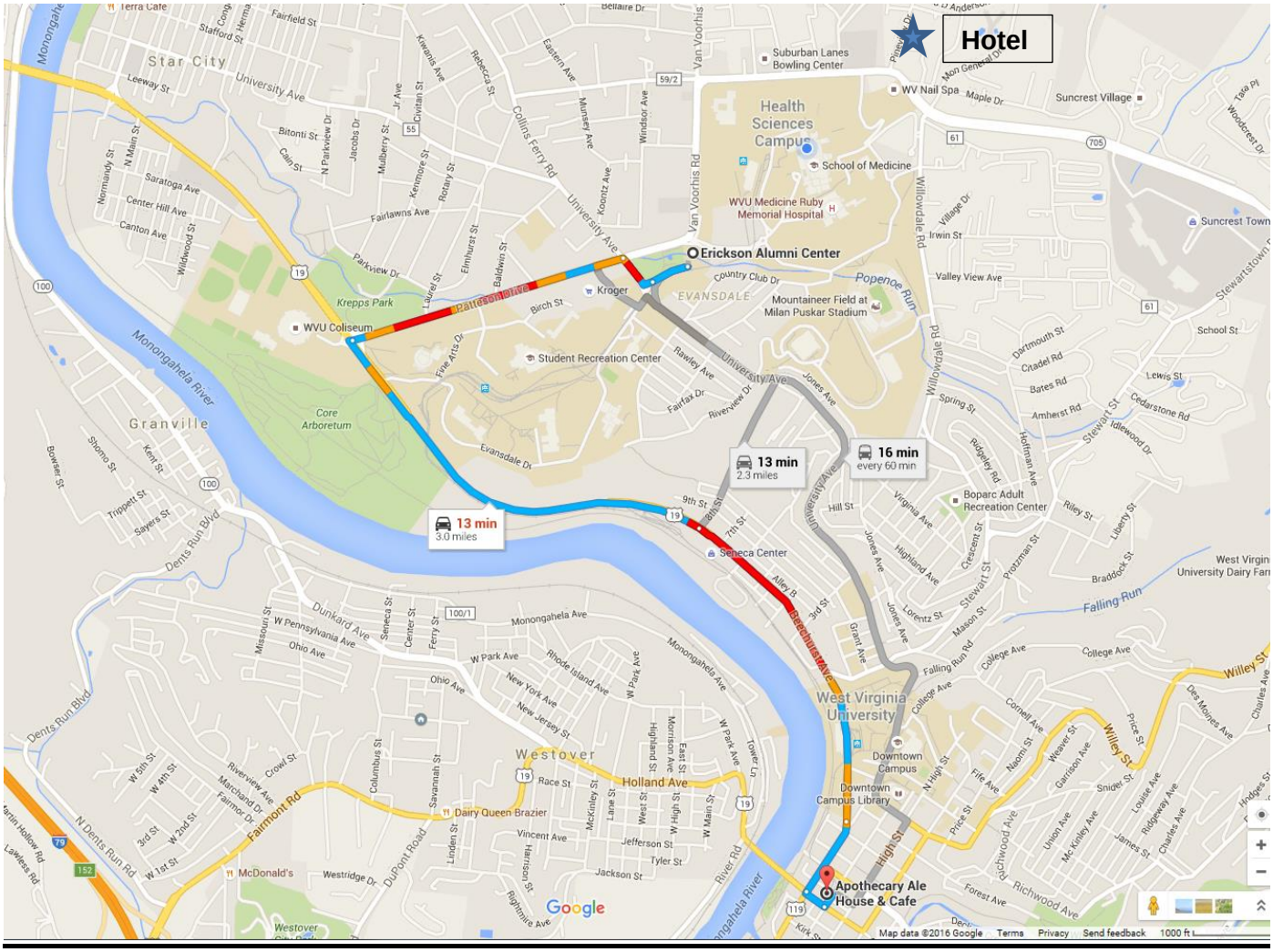
Sand added to fracking fluid that is pumped into the well bore is used as a proppant to maintain fissures in fractured shale to facilitate gas flow during unconventional gas drilling. Mechanical manipulation of the sand during preparation of fracking fluid gives rise to respirable dust (fracking sand dust; FSD) and inhalation exposures of workers to crystalline silica at levels in excess of regulatory standards. We characterized properties of FSD and some of its biological effects. Analysis of work site-derived FSD particles (≤2 μm major axis; >95% crystalline silica) using energy-dispersive X-ray spectroscopy (EDS) revealed eight categories of differing compositions based on the presence of Si, O, Al, Mg, K, Ca, Mn, Fe, and Co; elemental heterogeneity was present in individual particles. Inhalation of FSD by rats (6 h/d, 4 d, 10 or 30 mg/m^3) led to dose-dependent lung burdens at 1, 7, and 27 d post-exposure, with clearance noted following 1 d post-exposure. In enhanced dark field (EDF) images of trachea, particles associated with epithelium were observed 1 d but not 7 or 27 d post-exposure. EDF studies of clearance revealed that particles in the alveolar region of the lungs were reduced to ~50% and ~30% by 7 and 27 d post-exposure; none were present at 90 d post-exposure. Clearance of FSD appears to be more rapid than that reported for pure crystalline silica (i.e., MIN-U-SIL). Previously, inhalation of 30 mg/m^3 FSD was observed to inhibit tracheal epithelial active Na^+ transport, suggesting that the epithelium is a target of FSD effects. We, therefore, examined the effects of 18 h incubation with apical FSD (0.0001 – 1 mg) on cytokine and lactate dehydrogenase (LDH) release into apical and basolateral media from normal human bronchial epithelium in air-interface culture. Changes in cytokine levels in a 65-cytokine panel of growth factors and inflammatory mediators were modest (0.1 – 1 mg). No LDH was released into the basolateral medium; LDH released into the apical medium was unaffected by FSD. The results indicate that FSD is fairly rapidly cleared from the lungs following inhalation and is without appreciable toxicity to airway epithelial cells.

Group Dinner

Apothecary and Ale House Café
227 Chestnut St
Morgantown, WV 26505

Directions:

Take Patteson Drive to Monongahela Blvd (Left at the Coliseum)
Follow Monongahela Blvd (Turns to Beechurst) and turn left onto Pleasant St.
Parking can be found on the street, in the parking lot behind Huntington Bank, or in the garage by Black Bear Burritos.
The restaurant is located at 227 Chestnut Street, toward downtown and before the Walnut PRT station stop.



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NOTES