SANTA FE, NEW MEXICO, USA

AUGUST 28-31, 2022

13TH INTERNATIONAL PARTICLE TOXICOLOGY CONFERENCE

PROGRAM

WHAT DOES A SUCCESSFUL DOSIMETRY MODEL LOOK LIKE?

WILD FIRES, MICROPLASTICS, AND SYSTEMIC EFFECTS, OH MY!

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#IPTC2022

BRINGING PARTICULATE EXPERTS TOGETHER SINCE 1979


WELCOME
13TH INTERNATIONAL PARTICLE
TOXICOLOGY CONFERENCE

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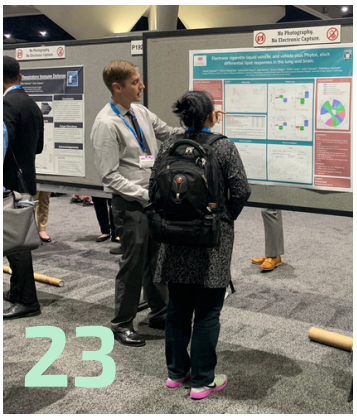
SCIENCE + NETWORKING + FOOD



ABSTRACTS

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DINING: RED OR GREEN?

Santa Fe is a culinary gem in the U.S. with a variety of Southwestern restaurants and other diverse options within walking distance from the hotel. We encourage attendees from near and far to enjoy their visit!

2022IPTC@SALUD.UNM.EDU

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GENERAL INFORMATION

MEET THE ORGANIZING COMMITTEE

MATTHEW CAMPEN, PHD
University of New Mexico, USA

ALEXANDRA NOËL, PHD
Louisianna State University, USA

FLEMMING R. CASSEE, PHD
RIVM, The Netherlands

ROEL SCHINS, PHD
The IUF, Germany

JARED BROWN, PHD
Colorado University, Anschutz, USA

CHRISTOPHER REILLY, PHD
University of Utah, USA

TERRY GORDON, PHD
New York University, USA

AARON D. ERDELY, PHD
CDC, NIOSH, HEL,D, PPPRB, USA

URMILA KODAVANTI, PHD
US EPA, USA

CUIQING LIU, PHD
Zhejiang Chinese Medical University, China

MICHAELA RITZ
University of New Mexico, USA

JESSICA BEGAY, MS
University of New Mexico, USA

EVENT ROOM LOCATIONS

Trainee Satellite Meeting (August 28)	*Santa Fe Room*
Pre-Conference Workshop (August 28)	*Sitha Room*
Welcome Reception	*Santa Fe Room*
Main Conference Sessions	*Lumpkins Ballroom*
Poster Sessions	*Perimeter of Lumpkins Ballroom and Mezzanine*
Exhibitors	*Mezzanine*
Lunches	*Garden Terrace*
IPTC Reception (August 30)	*La Terraza*

WELCOME TO THE 13TH INTERNATIONAL PARTICLE TOXICOLOGY CONFERENCE!

¡Bienvenidos a Nuevo Mexico! It is a pleasure to welcome so many friends – old and new – to the latest edition of the International Particle Toxicology Conference, here in Santa Fe, New Mexico, USA. I relocated to New Mexico 20 years ago and have loved living here, raising a family and pursuing a career in particulate toxicity research. I hope our attendees get a taste of the amazing landscapes, unique cultural features, and amazing cuisine of this region.

At the end of the 12th IPTC meeting in Salzburg in the fall of 2019, I was honored by the request to host the 13th IPTC in New Mexico in 2022. Of course, the pandemic events that occurred in the interim led to an abundance of second guessing my decision to take the reins of this event. But it has been clear over the past 3 years that particulate science remains as important as ever. We witnessed a surge in e-cigarette-related hospitalizations in 2019 in the US, followed by a global pandemic of a new virus that was carried by droplets. Wildfires also have been intense in recent years, with major outbreaks around the globe. And of course, we are at the cusp of understanding how life will exist with the emerging reality of microplastics, which have been found in the most remote regions of the planet, and deep in human tissues. So the scientific fervor around particulates is even greater today than it was in the last meeting!

Another major outcome from the pandemic has been a deficit in true networking. Virtual platforms have a role, and there are certainly great examples of scientific workshops conducted in the digital environment. But for trainees and junior faculty, the opportunity to really converse with the most influential scientists of our field and get feedback on their research is extremely hampered in virtual platforms. And, from the other perspective, established investigators have had trouble identifying new students and postdoctoral fellows to provide the brilliance and talent needed to fuel the research engine. Even the promotion of collaboration and team science is hindered without face-to-face interaction. During this year's IPTC, we really want to emphasize how essential in-person events are to career growth and scientific progress. The poster sessions, group dinner, and pre-meeting workshops are designed to promote this interaction.

Lastly, **I wish to acknowledge that our conference occurs on unceded ancestral Tewa Land, specifically O'gha Po'oge, now present-day Santa Fe. Indigenous descendants of numerous Puebloan, Diné (Navajo), and Apache communities reside throughout the region and are recognized as essential leaders and contributors to our culture and to the research community. We honor the land itself and those who remain stewards of this land throughout the generations and also acknowledge our committed relationship to Indigenous peoples.** I hope that during your stay in Santa Fe, you have a chance to visit some of the many museums and galleries that reflect the Native American influence and detail their history and culture.

Sincerely,

Matthew Campen, PhD, MSPH
University of New Mexico, College of Pharmacy
13th ITPC Organizing Director

CODE OF CONDUCT

The 2022 International Particulate Toxicology Conference (IPTC) is committed to providing a safe and productive environment for all of its meetings; one that fosters open dialogue, the free exchange of scientific ideas, the promotion of equal opportunity, and is free of any sort of harassment, coercion, and discrimination. All meeting participants are expected to treat others with respect and consideration, follow venue rules, and alert IPTC organizers of instances of harassment, coercion, or discrimination. The IPTC is fully cognizant that there are areas of our science that are controversial. Our meetings can and should serve as an effective forum to consider and debate scientifically-relevant viewpoints in an orderly, respectful and fair manner. The policies herein apply to all meeting attendees, speakers, exhibitors, guests, staff, contractors, and volunteers, and are based off of the University of New Mexico's (UNM) Visitor, Student, and Administrator Codes of Conduct (found in the UNM Student Handbook).

WHAT IS HARASSMENT?

Harassment includes speech or behavior that is not welcome or is personally offensive, whether it is based on ethnicity, race, gender, religion, age, body size, disability, veteran status, marital status, sexual orientation, gender identity, or other reason not related to scientific merit.

Behavior acceptable to one person may not be acceptable to others. As such, meeting attendees must use discretion to ensure that respect is clearly communicated. Harassment expressed in a joking manner still constitutes unacceptable behavior. Retaliation for reporting harassment is a violation of this policy, as is reporting an incident in bad faith.

REPORTING HARASSMENT

The 2022 IPTC is committed to providing a safe environment for everyone at any of its meetings. If an individual experiences or witnesses harassment of any kind, they should contact IPTC organizers at 2022IPTC@salud.unm.edu . All complaints will be treated seriously and responded to promptly.

If an individual wishes to file a formal grievance of harassment:

- The individual should notify meeting staff at 2022IPTC@salud.unm.edu
- 2022 IPTC staff will discuss any grievance first with the individual filing the grievance then with the alleged offender, seek counsel if the appropriate action is unclear, and report the incident and findings to the 2022 IPTC Committee, and the incident will be reported to the UNM Office of Equal Opportunity for further investigation.
- The 2022 IPTC will confidentially report instances to the UNM Office of Equal Opportunity and, also, consult with the individual filing the grievance before taking any action.

The 2022 IPTC organizers reserve the right to remove an individual from a meeting without warning or refund of any expenses, to prohibit attendance at future IPTC meetings, and to notify the individual's employer. If there are questions related to this policy, please contact the 2022 IPTC Director at 2022IPTC@salud.unm.edu.

ATTENDENCE TERM AND CONDITIONS

By registering for the 2022 IPTC, you are agreeing to abide by the 2022 IPTC Code of Conduct, the UNM Visitor Code of Conduct policy, and to the following terms and conditions, granting 2022 IPTC permission to:

- Reproduce, copy, and publish your image, voice, and any or all media taken as part of the International Meeting.
- Share your contact information with organizations that the 2022 IPTC believes might have a product or service of interest to you. Limited data provided to third parties include name, title, affiliation, and business address. Your telephone and fax numbers and email will not be disclosed to third parties.
- Share your name and affiliation with 2022 IPTC exhibitors and International Meeting Supporters.
- Include you in the attendee list, which includes your name and affiliation, accessible to meeting registrants using the 2022 IPTC Program (printed).

2022 IPTC registrants are prohibited from:

- Including distributing and/or posting promotional materials, special offers, job offers, product announcements, or solicitation for services outside of the 2022 IPTC and/or other designated spaces without specific permission from the IPTC Organizing Committee. 2022 IPTC reserves the right to remove such messages and potentially ban the sources of those solicitations.
- Capturing or copying of any aspect of the 2022 IPTC without the consent of the presenter(s)/author(s)/ exhibitor(s)/etc., including but not limited to slide presentations, video presentations, audio presentations, Q&As, chats, exhibits, posters, and abstracts.
- Causing a disruption to any meeting activity, session, or event.
- Sharing derogatory, offensive, or inappropriate content in any format during the course of the meeting.

These policies will be enforced by the 2022 IPTC. Those individuals who do not comply will be asked to leave the meeting. To request an exemption from any of the Annual Meeting policies, written notification by the registrant must be submitted to the 2022 IPTC Committee before the start of the International Meeting.

IPTC PRIVACY POLICY AND DISCLAIMER

For the duration of the UNM-hosted conference to be held in Santa Fe, NM, the 2022 IPTC adheres to the University of New Mexico's general privacy policy and information security disclaimers. For more detailed information, you may also visit the UNM Administrative Policies handbook.

COVID-19 POLICY

To protect the health and safety of all attendees, staff, and the Santa Fe community, all conference attendees with the ability to do so must be fully vaccinated against COVID-19, including available boosters. Thank you in advance for your help in keeping our colleagues and community safe!

Attendees who test positive for COVID-19 before or during the conference should notify the IPTC Organizing Committee at 2022iptc@salud.unm.edu to discuss opportunities for refunds.

If you show symptoms of or test positive for COVID-19 while attending the 13th IPTC, please contact the IPTC Organizing Committee and refrain from social contact. Two COVID-19 rapid-tests will be provided in attendee welcome packets, and the committee recommends that attendees use one of the provided tests prior to attending any conference events.

If you have any questions regarding these policies, please contact the 2022 IPTC office during event.

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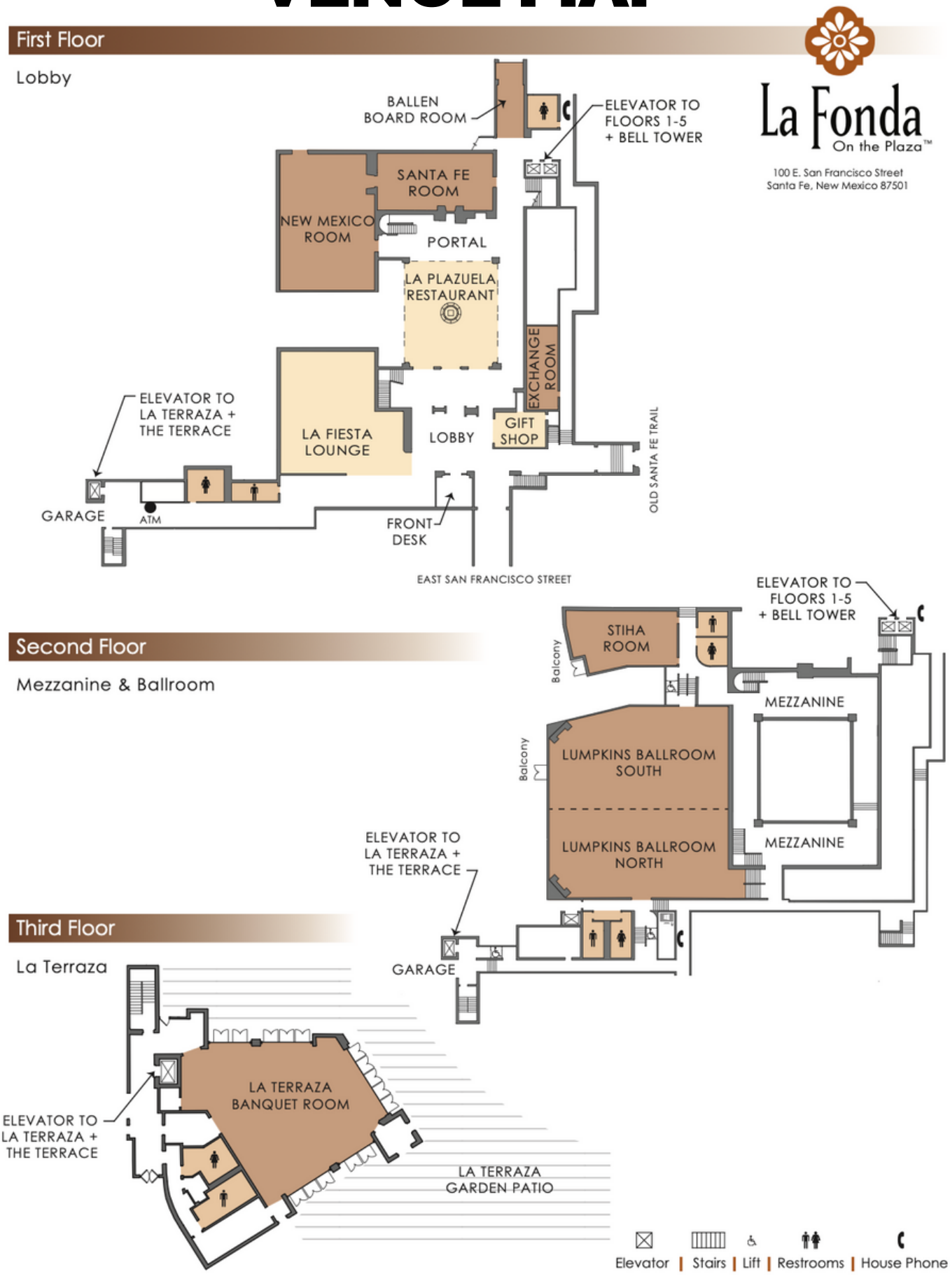
VENUE MAP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The University of New Mexico Center for Metals in Biology and Medicine is funded by the IDeA program of the National Institutes of Health (NIH) National Institute of General Medical Sciences (NIGMS), along with support from the UNM College of Pharmacy, the UNM Health Sciences Center, the UNM UNM Comprehensive Cancer Center, and the UNM Clinical and Translational Science Center. Funding for UNM's CMBM is through the **NIH NIGMS grant P20 GM130422**

Funding for this conference was made possible (in part) by **1R13 ES034641-01** from the National Institute of Environmental Health Sciences. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government.

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DINING IN SANTA FE: RED OR GREEN?

Meals in Santa Fe range from established fine-dining restaurants where you can get dressed up, to leisurely spots where you can arrive in hiking shorts after a day in the Sangre de Cristo mountains. No matter where you go, though, the entire city is suffused with the laid-back, can-do attitude of the Southwest. Whether you're looking for iconic home-style New Mexican cuisine or a high desert take on global cuisine, the City Different has it.

Reminder: Don't forgot to Google before you go! Some restauramts may open certain hours/days.

DOWNTOWN

The Shed 113½ East Palace Ave. (505) 982-9030

As the sister restaurant to La Choza, this one's got a good 30 years on its sibling and has been a local fave for every moment of that time. One of the city's culinary treasures since 1953, the restaurant is renowned for their legendary red chile and blue corn enchiladas. Download your Santa Fe Margarita Trail App and save on the signature Roca Coin Margarita (and collect a free Margarita Trail T-shirt once you earn five passport stamps). We strongly recommend reservations.

Market Steer Steakhouse 210 Don Gaspar Ave. (505) 992-6354

For the purpose of this listing, it's all about the steak, but you can add lobster tail or crab Oscar to those orders and enhance it all with house-made truffle butter.

The Bull Ring 150 Washington Ave. (505) 983-3328

Since debuting La Boca in 2006 in downtown Santa Fe, chef James Campbell Caruso has merged regional Southwestern ingredients with methodical Spanish technique, transporting eaters from New Mexico to Spain. For example, Caruso pairs small plates like trio de califlor — roasted cauliflower with harissa barbecue sauce and manchego cream — with an impressive selection of Mediterranean and South American wines.

Il Vicino 321 W San Francisco St. (505) 986-8700

Leaning into the deep Neopolitan flavors, Il Vicino is a paradiso della pizza. There are classic options like margherita and simple pepperoni, or you can get nuts and go for the gorgonzola-goat cheese-portobello mixed Bianca.

Upper Crust Pizza 329 Old Santa Fe Trail (505) 982-0000

This place has a dedicated following that's pretty impressive. That sunny chicken pesto pizza is a delight, as is the red chile New Mexico mix.

La Boca 72 W Marcy St. (505) 982-3433

Since debuting La Boca in 2006 in downtown Santa Fe, chef James Campbell Caruso has merged regional Southwestern ingredients with methodical Spanish technique, transporting eaters from New Mexico to Spain. For example, Caruso pairs small plates like trio de califlor — roasted cauliflower with harissa barbecue sauce and manchego cream — with an impressive selection of Mediterranean and South American wines.

Cafe Pasqual's 121 Don Gaspar Ave. (505) 983-9340

Instead of being doused in green chile sauce, the handheld breakfast burrito contains minimalist strips of uncut, roasted green chile peppers. For those seeking a purer commune with the New Mexican green chile, this is a good way to go.

Sazón 221 Shelby St, (505) 983-8604

The signature mole negro is subtle and balanced; the pork belly tacos are divine; and the sweeter-than-normal chiles en nogada, made with a jalapeno balsamic reduction, offer a new take on a classic. It's cooking like this — which rivals anything you might find on a white tablecloth in Mexico City — that keeps tables at this adobe-walled restaurant in high demand.

Coyote Cafe & Rooftop Cantina 132 W Water St. (505) 954-0320

Sunday afternoons on the patio may as well be the official Santa Fe pastime. The patio at Coyote Cantina is a perfect place for Sunday Funday. Check out the green chile infused Norteño and collect another stamp on your Margarita Trail Passport.

Del Charro 101 W Alameda St. (505) 954-0320

From margaritas with a little extra in the shaker, to a good list of beer and plenty of spirits, this hotel bar is also a local haunt. Plus, great pub food with reasonable pricing despite being downtown.

CANYON ROAD

Geronimo 724 Canyon Road (505) 982-1500

Pan-seared Alaskan halibut, green miso sea bass, mesquite grilled Maine lobster tails...we'd say more, but there's a chance you'll keel over. Geronimo tops the BOSF list yet again as the reigning champ of local fine dining. Executive chef Sllin Cruz rotates his menu seasonally, so this Canyon Road haunt easily becomes a year-round experience with changing options. Don't say we didn't warn you about a Geronimo obsession, though. It's a thing.

The Compound 653 Canyon Road (505) 982-4353

Chef Mark Kiffin can cook a mean schnitzel, and that organic stone ground polenta has a bit of an attitude, too. Best to eat it slowly to punish it, knowing that a butterscotch budino is on the way for dessert.

The Teahouse 821 Canyon Road (505) 992-0972

With an endless list of fogs and blooming teas, plus a humongous collection of greens and many more steaming and iced options, The Teahouse is Santa Fe's favorite tea destination. We're talking premium Earl Greys, wellness teas and oolongs. Don't skip the food menu, either, but when you're looking for as many teas as possible in one place, this Canyon Road mainstay will simply not be beat.

THE RAILYARDS

Paloma Restaurant 401 S Guadalupe St. (505) 467-8624

This upscale Mexican restaurant makes its tortillas from local landrace blue corn that's nixtamalized in house. The result is a complex flavor that elevates Paloma's tacos — topped with carne asada, sea bass, or cauliflower — into some of the best in town.

Second Street Brewery - Rufina Taproom 2920 Rufina St. (505) 954-1068

With three locations around town, Second Street Brewery is a standby among locals for burgers, beers, and live music. The company's newest outpost is the spacious Rufina Taproom, conveniently located near the popular immersive art space Meow Wolf for pre- or post-visit refreshment. You'll find the brewery's bread-and-butter staples alongside Cajun-inspired dishes by chef Milton Villarubi

Tomasita's 500 S Guadalupe St. (505) 983-5721

Most enchiladas are rolled, but Tomasita's serves its enchiladas flat. Chicken, cheese, beef, shrimp, or vegetables are sandwiched between moist but sturdy layers of yellow corn tortillas, which are smothered with green chile sauce and cheese.

Andiamo! 322 Garfield St. (505) 995-9595

Start with prosciutto and fresh mozzarella, then hop into the penne arrabiata that's topped with chile flakes. Go for grilled trout or crispy duck leg. Just hurry.

Opuntia 1607 Alcaldesa St. (505) 780-5796

A cute little garden paradise in the Rail- yard might not've been what you were expecting the space to become five years ago, but, man, are you satisfied with these results. Look out from the second-story patio and marvel.

Radish & Rye 505 Cerrillos Rd. (505) 930-5325

The menu focuses on local sourced foods and simple dishes executed flawlessly. In other words, Radish and Rye takes the "farm-to-table" concept to a whole new level. Additionally, the restaurant runs a top bourbon program, an extensive whisky list and cocktails featuring hand-selected barrels of Buffalo Trace.

Boxcar Bar and Grill 530 S Guadalupe St. (505) 988-7222

We swear by sports in the daytime, live music in the night time, and great food all the time.

WORTH THE DRIVE

La Choza Restaurant 905 Alarid St. (505) 982-0909

You know these margs are worth the long wait. We've got no clue why drinks taste so different here, but there's a certain liveliness that must affect the mix at La Choza. Maybe it's just the atmosphere, that knowledge you're gonna have a good time and a great meal. We're fans of the fruity Choza Red, but if you want to give building your own marg a try you can do that, too. It's like an art project in a glass that goes well with chile.

Maria's New Mexican Kitchen 555 W Cordova Rd. (505) 983-7929

Flip to the classics menu if you're nervous (the Cheap Date has saved us so much deciding time), but have no fear—the list is so massive you'll find something to love.

Jambo Cafe 2010 Cerrillos Rd. (505) 473-1269

Chef Ahmed Obo combines the Swahili, Indian, Arabic, and European culinary influences of Kenya's Lamu Island, where he was raised, to provide a unique taste of East African fare. At Jambo Cafe, opened in 2009, check out the coconut pili pili shrimp, which features wild shrimp over spicy coconut tomato stew and basmati rice, alongside fan favorites like grilled jerk chicken and vegetarian dishes.

India House 2501 Cerrillos Rd. (505) 471-2651

Chef Kewal Singh Dhindsa took his love of classic South Indian food from Los Angeles to New York to the Caribbean before settling in Santa Fe. At India House, where the dining room is expansive and the service immaculate, try anything from the tandoor oven, like the chicken wings broiled over mesquite and mixed with yogurt, garlic, and ginger. Don't be shy if you like heat — ask for recommendations, or alter your favorite dishes with additional spice. India House will go above and beyond to make you sweat.

Pantry Restaurant 1820 Cerrillos Rd. (505) 986-0022

One of Santa Fe's most beloved breakfast hotspots since 1948, the restaurant touts itself as "Santa Fe's Meeting Place." The menu offers a healthy mixture of traditional breakfast favorites and New Mexican breakfast staples, usually smothered in the region's signature red or green chile (for a little of both, ask for "Christmas").

Back Road Pizza 1807 Second St., Ste. 1 (505) 955-9055

Thin-crust lovers know where the good stuff is: Spoiler alert, it's here. Piper Kapin, owner and operator of the joint, has created a well-known cool space in Midtown where the pizza is fab and you can even shoot a game of pool sometimes. Back Road also became a de facto grocery during the height of the pandemic and kicked off Santa Fe's love affair with Detroit-style pizzas. Thin crust or deep dish, the point remains the same—you love 'em, Santa Fe.

The Chocolate Maven 821 W San Mateo Rd. (505) 984-1980

Look, it's possible after going through Chocolate Maven's myriad bakery options you might find something that doesn't work for you. What we're saying is it takes ages to get to that point. I mean, Cape Cod cranberry orange cookies? Mocha peanut butter Oreo cake? Espresso brownie bars?! We're getting a sugar rush just from writing this. And oh no: Honey cakes and tea loaves? OK, Maven, we get it. We surrender. We'll keep coming back.

Clafoutis 333 W Cordova Rd. (505) 988-1809

Why our little desert town has so much French-ness we'll never know, but we aren't ones to complain. While you face a parking lot rather than the Champs-Élysées, never doubt the power of Clafoutis' croissants to fly you back to your French memories.

Santa Fe Bite 311 Old Santa Fe Trl (505) 982-0544

The green chile burger is the dish to get at Santa Fe Bite. It's the massive hunk of beautifully seared meat that really makes this dish, but the green chiles add a welcome bit of mild heat and help moderate the richness of the beef.

Shake Foundation 631 Cerrillos Rd. (505) 988-8992

Where fresh burgers come with shoestring fries and eating outside on long picnic tables is considered cool, Brian Knox's joint feels like we're in Austin, minus the dreadful heat.

Santa Fe Brewing Company

The facility has indoor and outdoor venues hosting several nationally touring arts throughout the year. Known for their flagship IPAs as well as rotating seasonal offerings, the brewery is New Mexico's original and most successful craft brewery.

Izanami 21 Ten Thousand Waves Way (505) 982-9304

Leaping from third place last year into first this year, Izanami has traditionally been the place we go for that higher-end Asian cuisine—literally and figuratively, as it's up in the mountains. Find sake in abundance, plus herbs grown on-site, meaning the food is as fresh as fresh can be. Ask anyone on staff and they can tell you where the restaurant sources its beef, fish and whatever else is on the menu. Try grilled miso bass or kurobuta pork belly tacos, then finish off the meal with a passion fruit tart. Staff won't even mind if you show up in a robe.

****Reviews Provided by The Santa Fe Reporter 2022****

Trainee Satellite Meeting

AUGUST 28, 2022

Location: Santa Fe Room

Open to all registered trainees (postdoctoral fellows, graduate students, undergraduate students). Lunch is available to registered trainees. Abstarcts for Trainee Platform Presenations are found within the Poster Session Abstracts (pg. 23-55)

8:30	Convene, Registration	
8:45	Welcome, Opening Remarks	Alexandra Noël, LSU
9:00	The road traveled to predicting potential human health effects of carbon nanotubes & nanofibers	Aaron Erdely, NIOSH
9:30	Training/Workshop:Computational modeling for inhaled aerosols	Annie Jarabek, US EPA
10:45	Coffee Break	
11:00	Inhaled Aerosol Practicum: Application of the EPA MPPD Model for Interspecies Extrapolation and Risk Assessment: A Tale of Two Use Cases	Trainees' activity
12:45	Lunch	
1:45	Career Oriented Talk (20 min) + Q & A with trainees (10 min) Academia	Phoebe Stapleton, PhD, Rutgers University
2:15	Career Oriented Talk (20 min) + Q & A with trainees (10 min) Government	Aaron Erdely, PhD, NIOSH
2:45	Coffee Break	
	TRAINEES' PLATFORM PRESENTATIONS	
3:00	Role of Lipid Metabolism in Particulate Matter - Induced Lung Inflammation and Injury (10 min)	Hannah Lovins, OSU
3:15	Particulate Air Pollution and Viral Activation by Airway Proteases: Assessed by a Novel Assay in Human Nasal Epithelial Cells (10 min)	Stephanie Brocke, UNC
3:30	Dietary Supplementation with Omega - Fatty Acid Prevents Silica - Triggered Autoimmune Disease in Adult Lupus - Prone Mice (10 min)	Lauren Heine, MSU
3:45	Physicochemical Drivers of Primary Toxicologic Outcomes induced by Carbon Nanotubes and Nanofibers from U.S. Facilities (10 min)	Kelly Fraser, NIOSH
4:00	Coffee Break	
4:15	Magnetic Resonance Imaging Contrast Agents Induce Acute Tubular Damage and Gadolinium-rich Nanoparticle Formation in Renal Cortex (10 min)	Joshua DeAguero, UNM
4:30	Acrolein, a major constituent of combustion emissions, induces sexually dimorphic neuroendocrine and metabolic dysfunction (10 min)	Devin Alewel, US EPA
5:00	Awards announcement	Matthew Campen, UNM & Alexandra Noël, LSU
5:10	Concluding remarks, Adjournment	Alexandra Noël, LSU

Pre-Conference Workshop

AUGUST 28, 2022

Location: Stiha Room
Time: 4:30pm - 6:30pm
Topic: Animal models for air pollution need new approaches to reproducibility

Open to all registered attendees. Chairs for this workshop will be Caleb E Finch (USC) and Matthew J Campen (UNM).

Chronic exposure to elevated air pollution particles and gases is strongly associated with diseases of arteries, brain, heart, and lungs. Corresponding experimental animal models include exposure to ambient or concentrated air pollution particles, gases, and diverse subcomponents. Extensive data indicates shared biochemical and genomic responses of air pollution associated diseases. However, experimentalists are challenged by wide variations of ambient air pollution and its components by location and time, and different sources of sub-components, like diesel exhaust particles (DEP).

SPEAKERS:
Pamela J. Lein (UC Davis) Rodent models for exposure to traffic tunnel air pollution collected and delivered unchanged in real-time: strengths and limitations.
C. E. Finch and Kristina Shkirkova (USC): Diesel exhaust particles from NIST induce dose and time-dependent neurotoxic responses in mouse grey and white matter.
Joel Kaufman (U Washington): Freshly-generated, diluted, and aged diesel exhaust as an inhalation model for traffic-related air pollution: strengths and limitations."
Matt Campen: Photoaging of polystyrene microspheres causes oxidative alterations to surface physicochemistry and enhances airway epithelial toxicity.
Urmila Kodavanti (EPA): "Incorporating neuroendocrine mechanisms and resiliency in assessing air pollution health effects."
Ralf Zimmermann (University of Rostock) "Which chemical aerosol properties shall be measured in particle toxicological studies? Novel approaches for particle characterization to complement aerosol toxicological studies"

Open discussion: Jon Samet (U Colorado), CE Finch and Matt Campen
Approaches to shared standards for animal exposures. Should we consider a shared air pollution particle standard, modeled after the 'Research Cigarette' for tobacco toxicity.

Conference Day 1

AUGUST 29, 2022

Location: Lumpkins Ballroom

8:00	Convene, Registration, Breakfast	
8:40	Welcome, Opening Remarks	Campen, Brown, Gordon
RESEARCH SEMINARS: WILDFIRE-RELATED EXPOSURES AND HEALTH EFFECTS Chairs: Alexandra Noël & Matthew Campen		
9:00	Keynote – Comparative chemistry and toxicity of combustion emissions from biomass and synthetic materials	Ian Gilmour, PhD US Environmental Protection Agency, Research Triangle Park, North Carolina USA
9:40	Pre-existent stress increases susceptibility to ozone and wildfire smoke exposure	Thomas Jackson, US Environmental Protection Agency
10:00	Neurometabolomic Impacts of Modeled Wildfire Smoke Exposure in Aged Female C57BL/6 Mice	David Scieszka, University of New Mexico
10:20	Repeated Exposure to Wildfire Smoke Alters Pulmonary Gene and Metabolic Profiles in Male Long-Evans Rats	Katelyn Dunigan-Russell, The Ohio State University
10:40	Coffee Break	
RESEARCH SEMINARS: COMBUSTION-SOURCE PM Chairs: Flemming Cassee & Chris Reilly		
11:00	Invited Speaker – Particulate Exposure and Adverse Health Effects of Wildland Firefighting	Olorunfemi Adetona, PhD, Ohio State University
11:40	Sputum macrophage carbon load as a biomarker for episodic exposure to ambient combustion particles	Shuguang Leng, University of New Mexico
12:00	Effect of Combustion Particle Morphology on Biological Responses in a Co-culture of Human Lung Epithelial (A549) and Macrophage-like (THP-1) Cells at Air-liquid Interface Exposure Conditions	Kamaljeet Kaur, University of Utah
12:20	Exposure to eucalyptus smoke during sperm maturation alters motility and non-coding RNAs in caudal sperm	Colette Miller, US Environmental Protection Agency
12:40	Inhalation toxicity of copper sulphate pentahydrate and dicopper oxide	Craig Poland, Regulatory Compliance Limited, Edinburgh, UK
1:00	Paper of the Year – Particle and Fibre Toxicology	Flemming Cassee, Editor in Chief
1:05	Lunch - La Terraza	Provided by Hotel

Conference Day 1

AUGUST 29, 2022

Location: Lumpkins Ballroom

RESEARCH SEMINARS: PM COMPOSITION AND HEALTH EFFECTS Chairs: Marcus Garcia and Eliseo Castillo		
2:00	Invited Speaker – Is the placenta a barrier to microplastics?	Phoebe Stapleton, PhD, Rutgers University
2:40	Exposure and pulmonary toxicity assessments of dusts from machined plastic nanocomposites	Todd Stueckle, National Institute of Occupational Safety and Health, WV
3:00	Ultrafine particulate matter exposure aggravates pulmonary response to influenza infection during pregnancy	Natalie Johnson, Texas A&M University
3:20	Systemic Translocation of Polystyrene Microspheres: Quantitation Following Chronic Oral Gastric Exposure	Marcus Garcia, University of New Mexico
3:40	Invited Speaker – Evaluating indoor exposures to human respirable microplastic particles	Alison Elder, University of Rochester
4:10	Poster Session 1, jointly presented with MWSOT	Posters will be arranged on the perimeter of the Lumpkins Ballroom. They will be displayed all day, but attended from 4-6 pm for a dedicated viewing and social networking opportunity.
6:00	Dinner – free schedule Santa Fe is a culinary gem in the U.S. with a variety of Southwestern restaurants and other diverse options within walking distance from the hotel. We encourage attendees from near and far to enjoy their visit!	See our handpicked recommendations on pages 9-12

Conference Day 2

AUGUST 30, 2022

Location: Lumpkins Ballroom

8:00	Convene, Registration, Breakfast	
RESEARCH SEMINARS: SYSTEMIC HEALTH EFFECT OF PM Chairs: Urmila Kodavanti & Flemming Cassee		
9:00	Invited Speaker – The Potential Involvement of Inhaled Iron in the Neurotoxic Effects of Ultrafine Particulate Matter Air Pollution Exposure on Brain Development in Mice	Marissa Sobolewski, Ph.D. University of Rochester
9:40	A Case for Amorphous Silica Exposure in the Development of Chronic Kidney Disease of an Unknown Etiology	Keegan Rogers, University of Colorado
10:00	Nanomaterial induced effects in the healthy vs. diseased liver	Ali Kermanizadeh, University of Derby
10:20	Understanding the Lung-Gut Axis by Modeling the Influence of Genetic Diversity, Welding Fume Inhalation Exposure, and Lifestyle, on the Profile of Gut	Vamsi Kodali, National Institute of Occupational Safety and Health, WV
10:40	Coffee Break	
11:00	Emulating near-roadway exposure to traffic-related air pollution via real-time emissions from a major freeway tunnel system	Keith Bein, University of California, Davis
11:20	The effects of chronic exposure to ambient traffic-related air pollution on Alzheimer's disease phenotypes in wildtype and genetically predisposed male and female rats	Pamela Lein, University of California, Davis
11:40	The effects of traffic-related air pollution exposure on Alzheimer's disease-like pathology in mice	Roel Schins, IUF-Leibniz Research Institute for Environmental Medicine
12:00	Neurotoxic Responses to Diesel Exhaust Particle NIST 2975	Kristina Shkirkova, University of Southern California
12:20	Bridging the Pulmonary Toxicity of Inhaled Vapor/Liquid and Solid Particle-like Phases	Prof. Jürgen Pauluhn
12:40	Who put gases (aldehydes) in my particles? Electronic cigarette-derived particles (and gases) in cardiovascular and pulmonary toxicity	Daniel Conklin, University of Louisville
1:00	Lunch - La Terraza	Provided by Hotel

Conference Day 2

AUGUST 30, 2022

Location: Lumpkins Ballroom

RESEARCH SEMINARS: EMERGING SCIENCE, NOVEL APPROACHES **Chairs: Aaron Erdely & Terry Gordon**		
2:00	Invited Speaker – Evidence Integration for Risk Assessment of Inhaled Aerosols: Coupling Complimentary Experimental Work and Computational Workflows to Advance an Inhalation Integrated Approach to Testing and Assessment (iIATA)	Annie Jarabek, USEPA
2:40	Lipid Mediated Exacerbation of Nanoparticle-Induced Pulmonary Inflammation	Jonathan Shannahan, Purdue University
3:00	Cholesterol and particle-induced lysosomal membrane permeability	Rebekah Kendall, University of Montana
3:20	Serum Peptidome: Diagnostic Window into Pathogenic Processes following Occupational Exposure to Carbon Nanomaterials	Andrew Ottens, Virginia Commonwealth University
3:40	Invited Speaker – Nanoparticle modulation of pulmonary immune responses to inhaled allergens	Jamie Bonner, North Carolina State University
4:10	Poster Session 2	Posters will be arranged on the perimeter of the Lumpkins Ballroom. They will be displayed all day, but attended from 4:10pm-6 pm for a dedicated viewing and social networking opportunity.
6:00	IPTC Banquet – La Terraza	Southwestern-themed dinner for all attendees will be provided in the indoor-outdoor La Terraza facility at the La Fonda Hotel

Conference Day 3

AUGUST 31, 2022

Location: Lumpkins Ballroom

8:00	Reconvene, Introduction of Speaker	Jared Brown
RESEARCH SEMINARS: GLOBAL PM ISSUES Chairs: Jared Brown & Roel Schins		
8:20	**Keynote – A Panorama of Airborne Particles: From Then to Now and From Local to Global**	**Jon Samet, MD, University of Colorado School of Public Health**
9:00	Endotoxin exacerbates the NLRP3-dependent inflammatory potency of Saharan dust	Gerrit Bredeck, IUF – Leibniz Research Institute for Environmental Medicine
9:20	Characterization and in vitro toxicology of Subway PM	David Luglio, New York University
9:40	Heavy metal-laden particulate exposure induces enteroendocrine expansion in adult human colonoids	Roger Atanga, University of New Mexico
10:00	Coffee Break	Mezzanine
10:20	Are ultrafine particles from aircraft of more concern compared to those from road traffic?	Flemming Cassee, RIVM
10:50	Pulmonary Toxicity of Particulate Matter Emitted from Firearms	Ian Gilmour, US Environmental Protection Agency
11:10	Interactive outcomes after ultrafine particle and ozone mixed inhalation exposures	Salik Hussain, West Virginia University
11:30	The Importance of a Multidisciplinary Approach in Inhalation Toxicology	Danielle Carlin, NIEHS
11:50	In Memorium – Remembering Jon Hotchkiss, Renaud Vincent, and Bruce Boecker	Roger McClellan
12:10	**Concluding remarks, Student Awards**	**Matthew Campen, Alexandra Noël**
12:30	**Adjourn**	

Poster Session 1

AUGUST 29, 2022

Held with the Mountiain West SOT Conference

#1 Magnetic Resonance Imaging Contrast Agents Induce Acute Tubular Damage and Gadolinium-rich Nanoparticle Formation in Renal Cortex
Joshua DeAguero

#2 Sugarcane Ash and Sugarcane Ash - Derived Silica Nanoparticles Alter Mitochondrial Function and Metabolic Activity in Human Proximal Tubular Kidney Cells
Arthur Stem

#3 Acrolein, a major constituent of combustion emissions, induces sexually dimorphic neuroendocrine and metabolic dysfunction
Devin Alewel

#4 Direct Evidence of Metabolic Interactions between PBDEs and Gut Microbes: an In Vitro Metabolomics Study
Haiwei Gui

#5 Quantifying Heavy Metals in Interstitial Fluid
Robert Taylor

#6 Dietary Modulation of Pulmonary Lipids Influencing Toxicological Responses to Ozone Inhalation in Mice
Russell Hunter

#7 Combustion Derived Particulate Matter Exposure Exacerbates Influenza Infection by Inhibiting IL22 Production in Newborn Mice
Avinash Kumar

#8 Dietary Supplementation with Omega-Fatty Acid Prevents Silica-Triggered Autoimmune Disease in Adult Lupus-Prone Mice
Lauren Heine

#9 Particulate Air Pollution and Viral Activation by Airway Proteases: Assessed by a Novel Assay in Human Nasal Epithelial Cells
Stephanie Brocke

#10 Particulate Air Pollution and Viral Activation by Airway Proteases: Assessed by a Novel Assay in Human Nasal Epithelial Cells
Karol Dokladny

#11 Diesel Exhaust Particle Exposure Induces Polarization-State Dependent Functional and Transcriptional Changes in Monocyte-Derived Macrophages
Timothy Smyth

#12 Ex vivo to in vivo extrapolation for respiratory toxicity of inhalable substances
Katharina Schwarz

#13 Evaluation of WS1442 on Systemic Inflammation and Blood Brain Barrier
Raul Alejandro Salazar

Poster Session 2
AUGUST 30, 2022

#1 Comparative health effects in male and female mice exposed to Libby Amphibole Asbestos
Ujjwal Adhikari

#2 The Influence of Variable Electronic Cigarette Coil Power on Lung Cell Health
John Adragna

#3 Characterization of different aerosols using a novel thermal spray coating and inhalation exposure system
James Antonini

#4 Inhalation of combustion-derived environmentally persistent free radicals causes vascular endothelial injury mediated via AHR activation in alveolar type-2 pneumocytes
Ankit Aryal

#5 The effects of photochemical aging and interactions with secondary organic aerosols on cellular toxicity of combustion particles
Reuben Attah

#6 Surface engineering strategy to reach a safer and more performing profile of TiO2-NPs as sunscreen UV filters
Elena Cesa

#7 Effect of UV-Exposure on the Uptake and Toxicity of Respirable Polyester Fiber Particles
Eliane El Hayek

#8 Physicochemical Drivers of Primary Toxicologic Outcomes induced by Carbon Nanotubes and Nanofibers from U.S. Facilities
Kelly Fraser

#9 Single Cell- and Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry Techniques for Studying the Uptake of Gadolinium Based Contrast Agents in Chlamydomonas reinhardtii Algae and Arabidopsis Thaliana
Tyler Herek

#10 New Approach Methodologies for the hazard assessment of nanocellulose (NC): Tier 1 Testing - High Content Analysis Based Evaluation of the Toxicity of NC Materials in Human Intestinal and Macrophage Models
Kevin Hogeveen

#11 Comparison of Biological Response between Submerged, Pseudo-Air-Liquid Interface , and Air-Liquid Interface Exposure of A549 and Differentiated THP-1 Co-cultures to Combustion-Derived Particles
Kerry Kelly

#12 In vivo and in vitro toxicity of a stainless-steel aerosol generated during thermal spray coating
Vamsi Kodali

#13 Role of Lipid Metabolism in Particulate Matter-Induced Lung Inflammation and Injury
Hannah Lovins

#14 Changes in Protein Structure and Assembly with Fluoride Nanoparticles and Coexisting Ions
Ume Masakazu

#15 Particulate Toxicology in the 21st Century: Organotypic Models Reveal Trans-Epithelial Effects of Particulates in the Respiratory Tract
Shaun McCullough

#16 Genomic basis for individual differences in susceptibility to the neurotoxic effects of diesel exhaust
Alexandra Noël

Poster Session 2
AUGUST 30, 2022

#17 Identification of potential mediators implicated in the carcinogenicity of poorly soluble low toxicity particles in rats
Laeticia Perez

#18 Inhalation toxicity of copper sulphate pentahydrate and dicopper oxide
Craig Poland

#19 Examination of the exposome in an animal model: the impact of high fat diet and rat strain on local and systemic immune markers following occupational welding fume exposure
Katie Roach

#20 In vivo Lung Toxicity associated with Boron Nitride Nanotubes with Different Purities
Jenny Roberts

#21 Exposure to Arsenic and Uranium in Drinking Water Alters Gastrointestinal Health
Aaron Romero

#22 Aerosol-related particle dosimetry: Novel approach for generation of complex aerosol atmospheres for nano-particle dose metric studies
Katharina Schwarz

#23 Aerosol-related particle dosimetry: an experimental in vitro inhalation approach for studying biological effects as a result of defined modification of particle number- and mass-based dose-metrics
Katharina Schwarz

#24 Inflammation and transcriptome changes induced by asbestiform fibers in mice are ameliorated by a small molecule synthetic
Kinta Serve

#25 Inhaled Polyamide Particles Reduce Uterine Vascular Reactivity in Virgin Rats
Talia Seymore

#26 Nearly Free Surface Silanols Dictate Membrane Disruption That is Attenuated by Cholesterol Content
Matt Sydor

#27 Identification of Laboratory Animal Microplastic Consumption: A Quantitative Approach
Rachel Templeton

#28 QCL-Based Spectroscopy for Rapid Identification of Microplastics
Louis Tisinger

#29 Graphic Warning Labels Potentially Reduce Genotoxicity in Buccal Epithelial Cells Associated with Waterpipe Smoking
Chieh-Ming Wu

#30 MacLEAP: Machine-Learning Approach for Recognition and Quantification of Carbon Content in Airway Macrophages
John Yu

#31 Toxicological impact of Secondary Organic Aerosol compounds in air-liquid-interface exposed lung cell models
Ralf Zimmermann

#32 Which chemical aerosol properties shall be measured in particle toxicological studies? Novel approaches for particle characterisation to complement aerosol toxicological studies
Ralf Zimmermann

KEYNOTE SPEAKERS

COMPARATIVE CHEMISTRY AND TOXICITY OF COMBUSTION EMISSIONS FROM BIOMASS AND SYNTHETIC MATERIALS

Ian Gilmour, PhD
US Environmental Protection Agency, Research Triangle Park, North Carolina USA

Exposure to smoke from combustion of biomass (oak, eucalyptus, pine, peat) or synthetic materials (plastic, plywood cardboard) is associated with health effects including reduced respiratory function and increased pulmonary inflammation, although differences attributable to fuel types and combustion conditions, and their subsequent chemistries have not been well defined. We have developed a laboratory based furnace system that can combust different fuels or other flammable substrates under flaming or smoldering conditions with subsequent inhalation delivery to mice. Smoke emissions are extensively analyzed for a suite of chemicals and health effects are monitored following a one hour exposure to either the whole or filtered smoke. Pulmonary function is measured before, during and after inhalation exposure and numerous markers of pulmonary injury and inflammation are assessed either 4 or 24 hours after exposure. Condensates from these combustion emissions are also tested for mutagenic potential in the "Ames assay" and the potency compared either by relative mass of condensate or by calculated emission factor. We found that of the biomass fuels, eucalyptus and peat had more potent effects on pulmonary function and inflammation that the other fuels (oak and pine) and these effects were amplified with exposure to flaming smoke. Plastic had the most potent effects of the synthetic materials combusted and again flaming conditions presented the highest effects. Filtration in general ameliorated the health impact (lung function and inflammatory biomarkers) but to different degrees depending on the fuel. The mutagenicity results mirrored the pulmonary phenotypes with flaming plastic being the most potent and flaming biomass also having greater effects than smoldering smoke, and these effects were in general associated with increased levels of polyaromatic hydrocarbons. Finally, data from these studies are being applied in non-targeted clustering algorithms to understand how different chemical signatures or classes are associated with distinct phenotypic endpoints. This approach provides a good platform for pulmonary toxicity testing of different combustion emissions and endpoints are being compared to high throughput testing of cell based systems in the hope that this predictive toxicity testing may be validated and replaced with non-animal testing systems. (This abstract does not represent U.S. EPA policy;)

A PANORAMA OF AIRBORNE PARTICLES: FROM THEN TO NOW AND FROM LOCAL TO GLOBAL

Jonathan M. Samet MD, MS
Colorado School of Public Health

Airborne particles have long posed a global public health threat, causing epidemics of death during such notorious episodes as the London Fog of 1952 and contributing to an enormous burden of avoidable morbidity and mortality worldwide. Decades of research have deepened our understanding of how inhaled particulate matter (PM) causes injury and disease and brought new tools for investigating PM: small and accurate monitors for capturing personal exposure, models for estimating PM concentrations across the world, and in-vitro methods for investigating pathways of injury by particles. Over the decades since the mid-20th century, epidemiological research has causally linked exposure to PM indoors and outdoors to an ever-growing list of adverse health outcomes, extending from early life effects to premature mortality. The resulting burden of disease estimates are staggering, amounting to millions of lives lost prematurely. The epidemiological evidence in combination with deeper insights from toxicological and exposure sciences have been the basis for ever lower standards and guidelines for ambient PM concentrations. However, critical questions remain unanswered. Most significantly for supporting targeted control of the most critical PM sources, we have yet to have sufficient understanding of the PM characteristics that drive particle toxicity for the various adverse health effects of exposure and cannot link critical characteristics to sources. This presentation provides a historical perspective on the emergence of the evidence on PM and health, covering critical advances and their consequences. It then turns to the present, covering the profile of PM exposure globally and the associated burden of morbidity and premature mortality. It ends with a look to the future, covering research needs to provide evidence that will support further control of PM globally.

(This presentation does not involve animal and/or human research directly)

INVITED SPEAKER ABSTRACTS

EVALUATING INDOOR EXPOSURES TO HUMAN RESPIRABLE MICROPLASTIC PARTICLES

Alison Elder, PhD
University of Rochester

Plastics and their breakdown products are ubiquitously present in the environment from a variety of sources. While much attention has been focused on plastics in water, air sampling has also revealed the presence of fibrous and fragmented plastic particles in a wide range of sizes from the submicrometer scale up to tens of micrometers in length and diameter. Few studies have addressed the possible health consequences associated with exposures to airborne microplastic particles, but limited toxicological findings suggest they are not completely benign if they gain access to the lungs. Little is understood, though, about the degree to which these airborne plastic particles, specifically microplastics (0.1 mm–5 mm), can enter the respiratory tract, where they will deposit, and how polymer chemistry and morphology may be linked to adverse health outcomes following exposure. Whether microplastics behave in lung and secondary target tissues like other particle types or have unique toxicological properties is also an open question. Our work has sought to address critical questions about the inhalability and respirability of airborne microplastics in the indoor environment via particle size-restricted sampling coupled with morphological assessment and estimation of plastic burdens via Nile red staining. We found Nile red-positive (putative plastic) particles in air samples from indoor environments (office, campus/household laundry rooms, 3-D printing/engineering laboratory), most of which appeared to have a fragmented morphology. These particles represented ~1% of the total sample. Importantly, these particles were also found when sampling was restricted to the human respirable fraction, suggesting that they can reach the gas exchange region. Challenges remain in identifying the specific polymers in these air samples, whether they are equally distributed across all particle sizes, and whether indoor and outdoor microplastic particles are similar in terms of morphology, size, and chemistry.

EVIDENCE INTEGRATION FOR RISK ASSESSMENT OF INHALED AEROSOLS: COUPLING COMPLIMENTARY EXPERIMENTAL WORK AND COMPUTATIONAL WORKFLOWS TO ADVANCE AN INHALATION INTEGRATED APPROACH TO TESTING AND ASSESSMENT (IIATA)

Annie Jarabek
U.S. Environmental Protection Agency

Evidence integration for risk assessment of inhaled aerosols must evaluate data across a range of experimental platforms, which now include novel approach methods (NAMs), in vivo lab animal studies, and human clinical or epidemiological studies. Further, these NAMs may deploy in vitro submerged or air-liquid interface (ALI) exposure systems with various types of cell or tissue culture components. As emerging technology and research rapidly expands scientific knowledge of exposure and toxicity pathways, transparent workflows for the collection, alignment, and integration of exposure and effects data will be crucial for using this information effectively, credibly, and reliably for risk assessment. For evidence integration to be achieved coherently, risk evaluations must use exposure alignment as defined by the NASEM in its 2017 report Using 21st Century Science to Improve Risk-Related Evaluations, which accounts for determinants of dosimetry in different experimental systems using computational models or adjustments. Particle dosimetry models have been successfully used as the basis of size-selective exposure sampling and to improve interspecies extrapolation by accounting for differences in airway architecture, breathing modes and ventilation rates; and can likewise inform considerations of intra-human variability or susceptibility due to differences in these same parameters across age or with disease state. This presentation focuses on how dosimetry modeling now features as the critical link between exposure and response measures at various levels of observation (e.g., molecular, cellular, tissue) in a new conceptual, mechanistic construct for source-to-outcome characterization for risk assessment. The conceptual construct is based on coupling the aggregate exposure pathway (AEP) and adverse outcome pathway (AOP) frameworks. The construct serves as a mechanistic scaffold that creates context for development of an inhalation integrated approach to testing and assessment (iIATA). Dosimetry modeling plays a critical role in the iIATA by providing for the integration of physicochemical properties with exposure system and physiological parameters to translate exposures to internal dose across various experimental platforms. Coupling computational dosimetry approaches with targeted experimental work is critical to inform dose metric considerations, facilitate inferences regarding effect measures, and increase understanding of pathogenesis processes in support of the iIATA. Experimental work requisite to advance the application of NAMs in the iIATA includes how well a given NAM may characterize key events (KE) of pathogenesis in the respiratory tract or systemic tissues and the relationship of acute assays to chronic sequelae and adverse outcomes. Reporting standards for experimental work to ensure data are accessible to new computational work flows such as systematic review now routinely used in risk assessment are necessary and will be discussed. Agency efforts to ensure the availability of modular, interoperable computational dosimetry models are also highlighted. (The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.)

INVITED SPEAKER ABSTRACTS

NANOPARTICLE MODULATION OF PULMONARY IMMUNE RESPONSES TO INHALED ALLERGENS

Jamie Bonner PhD
North Carolina State University

The prevalence of asthma has markedly increased over the past several decades and exacerbation by ultrafine air pollution particles (i.e., nanoparticles) is a major problem that increases the severity of disease progression. Individuals with asthma therefore represent a susceptible population at high risk for the potent adjuvant-like properties of inhaled nanoparticles. Both ambient air pollution nanoparticles and engineered nanoparticles are thought to cause cellular injury through generating oxidative stress. However, an understanding of the molecular and cellular mechanisms through which nanoparticles and common allergens coordinately act to promote chronic allergic lung disease is lacking. Experimental evidence has shown that ambient air pollution nanoparticles or engineered nanoparticles exert strong pro-inflammatory and pro-fibrotic adjuvant effects in mouse models of allergic lung disease. For example, studies in mice show that chronic airway disease caused by common allergens, such as those from house dust mites (HDM), are synergistically increased by multi-walled carbon nanotubes (MWCNTs), a prototypical engineered nanoparticle that has broad applications in engineering. Airway fibrosis and mucous cell metaplasia are important pathological features of chronic airway disease during the progression of asthma that contribute to reduced lung function. Moreover, MWCNTs synergistically increase mRNAs encoding HDM-induced mediators of eosinophilia (e.g., CCL11) and fibrosis (TGF-b1, CCL2, Col1A1) in the lungs of mice. We propose a mechanism of nanoparticle exacerbation of chronic airway disease mediated by the adsorption of proteolytic HDM allergens to the surface of MWCNTs to form an 'allergen corona'. New evidence shows that HDM allergens in the corona have increased proteolytic activity and activate the protease-activated receptor-2 (PAR2) on lung macrophages. Triggering of PARs has been implicated in M2-like 'pro-fibrotic' polarization of macrophages, a process that is regulated by STAT transcription factors and arginase-1 (Arg-1). Interestingly, PAR2 deficiency in mice reduced airway fibrosis and decreased Arg-1 protein in lung that was amplified by co-exposure to HDM extract and MWCNTs. Collectively, the exacerbation of chronic allergen-induced airway disease in mice by nanoparticles appears to be regulated by a complex mechanism that involves modulation of the proteolytic activity of allergens through corona formation and amplification of pro-fibrotic cell signaling pathways that are mediated in part by PAR2. These findings have broad implications for asthma exacerbation in humans that are caused by a wide variety of ambient and indoor air pollution particles.

THE POTENTIAL INVOLVEMENT OF INHALED IRON (FE) IN THE NEUROTOXIC EFFECTS OF ULTRAFINE PARTICULATE MATTER AIR POLLUTION EXPOSURE ON BRAIN DEVELOPMENT IN MICE

Marissa Sobolewski, PhD
University of Rochester

Air pollution has been associated with neurodevelopmental disorders in epidemiological studies. In our studies in mice, developmental exposures to ambient ultrafine particulate (UFP) matter either postnatally or gestationally results in neurotoxic consequences that include brain metal dyshomeostasis, including significant increases in brain Fe. Since Fe is redox active and neurotoxic to brain in excess, this study examined the extent to which postnatal Fe inhalation exposure, might contribute to the observed neurotoxicity of UFPs. Mice were exposed to 1 µg/m3 Fe oxide nanoparticles alone, or in conjunction with sulfur dioxide (combined Fe (1 µg/m3) and SO2 as it has been shown to enhance Fe uptake; (SO2 at 1.31 mg/m3, 500 ppb) from postnatal days 4-7 and 10-13 for 4 hrs/day. Overarching results included the observations that combined Fe + SO2 produced greater neurotoxicity than did Fe alone, that females showed greater vulnerability to these exposures than did males, and that profiles of effects differed by sex. Both Fe only and combined Fe +SO2 exposures altered correlations of Fe and of sulfur (S) with other metals in a sex and tissue-specific manner, consistent with metal dyshomeostasis. Specifically, altered metal levels in lung, but particularly in frontal cortex were found, with reductions in metal correlations produced by Fe in females, but increases with other metals produced by Fe +SO2 in males. At PND14, marked changes in brain frontal cortex and striatal neurotransmitter systems were observed, particularly in response to Fe +SO2 as compared to Fe only, in glutamatergic and dopaminergic functions, changes that were of opposite directions by sex. Alterations in markers of trans-sulfuration in frontal cortex were likewise opposite in direction in females as compared to males. Residual neurotransmitter changes were limited at PND60. Increases in, serum glutathione and Il1-a and altered locomotor behavior were female-specific effects of Fe +SO2. Collectively, these findings suggest a role for the Fe contamination of air pollution in the observed neurotoxicity of ambient UFPs. They also underscore the importance of chemical speciation of metals to neurotoxic effects. Translation of such results to humans requires verification, and, if found, would suggest a need for regulation of Fe in air for public health protection.

NIH R01 ES032260 and R35 ES031689.

INVITED SPEAKER ABSTRACTS

PARTICULATE EXPOSURE AND ADVERSE HEALTH EFFECTS OF WILDLAND FIREFIGHTING

Olorunfemi Adetona, PhD
Ohio State University

Exposure of wildland firefighters to wildland fire smoke is more intense and frequent compared to the general population. Moreover, looser protective clothing compared to structural firefighters and little or no respiratory protection are typically worn during wildland firefighting. In addition, the evidence suggests heterogeneity in their particulate matter exposure at the fireline during wildland firefighting. However, there is lack of research about the contribution of dermal absorption to their occupational wildland fire smoke exposure. Also, the available evidence about the specific contributions of wildland fire smoke and its particulate component/composition to the adverse health effects of wildland firefighting is limited. The occupational health research among wildland firefighters so far has been focused on acute sub-clinical respiratory and systemic responses, with the observed physiological responses often being muted relative to the elevated smoke exposure possibly due to the healthy worker effect. Moreover, the long-term health impact of cumulative wildland fire smoke exposure in wildland firefighters has not been comprehensively investigated despite increasing number of wildfire deployments and applications of prescribed burning for land management. Nonetheless, filling the identified research gaps is essential for developing approaches to control exposure and mitigate the health effects this increasingly needful occupation.

IS THE PLACENTA A BARRIER TO MICROPLASTICS?

Phoebe Stapleton, PhD
Rutgers University

Micro-and nanoplastic particles have recently been identified as an emerging xenobiotic particle of toxicological interest. Polymers have been in production for commercial uses since the 19th century, with exponential commercialization, industrial and domestic use in the 20th century. Microplastics (< 5 mm in a single dimension) can be either intentionally produced as bulk materials or abrasives or through product degradation, the smallest fraction of these are identified as nanoplastics (< 100 nm [laboratory] or < 1000 nm [environmental]studies). Microplastic particles have been identifiedin food,beverages,water, and airborne samples; therefore, human exposures through inhalation and/oringestion routesis highly likely.Our laboratory investigates the toxicological consequences of xenobiotic particles exposure in a maternal-fetal model. In thestudies presented here, we exposed pregnant Sprague-Dawley rats to 20or 25nm polystyrene beads at gestational day 19 of pregnancyvia pulmonaryor gastric exposure.24-hour later, rats were sacrificed and tissues were prepared for analyses. Using Cytovivadark field microscopy, weidentified nanosized polystyrene particles in placentaland fetal tissues, indicating that these particles can breach initial biological barriers of the original exposure route (i.e., pulmonary or gastric epithelium) and translocate to and breachthe placental barrier.These results beget concerns for plastic particle depositionleading toimpaired placental functionand fetal support,localfetal-particleinteractionsduringin uterodevelopment,andlifelong concernsresulting in adult disease.

Note: Animals were housed in Rutgers AAALAC-accredited facilities and all techniqueswere approved by Rutgers IACUC.

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SEMINAR ABSTRACTS

Heavy metal-laden particulate exposure induces enteroendocrine expansion in adult human colonoids

Roger Atanga[1], Adrian Brearley[2], Eliseo F. Castillo[1], Matthew J. Campen[3], Julie G. In[1]
[1]*Division of Gastroenterology, Department of Internal Medicine, University of New Mexico Health Sciences Center;* [2]*Earth and Planetary Sciences, College of Arts and Sciences, University of New Mexico;* [3]*Department of Pharmaceutical Sciences, University of New Mexico Health Sciences Center*

Chronic heavy metals exposure has been associated with intestinal inflammation and higher incidences of colorectal cancer from observational studies, but the direct transcriptomic effect of heavy metals on intestinal epithelia is largely unknown. The goal of this study is to characterize particulate dust (primarily containing non-fissile uranium) as an environmental toxicant that damages intestinal epithelia and determine the transcriptomic changes that develop as a response. Human colonoids derived from healthy adult colonic biopsies (IRB approved study 18-626 and 18-171) were established and grown in Matrigel to physiologically model the intestinal epithelia. Particulate dust obtained near Jackpile uranium mine containing non-fissile uranium was added to colonoids for up to 24h. Control and dust-exposed colonoids (n=3 donors) were dissociated into single cells and processed for droplet-based single cell sequencing (scRNA-seq). Downstream analysis was validated in colonoids via immunostaining, immunoblotting, and ELISAs. scRNA-seq identified significant changes in the secretory lineage in uranium dust-exposed colonoids. Specifically, there was a 10-fold expansion of enteroendocrine cells (EEC) with increases in EEC hormones serotonin and PYY in all colonoid lines. Proliferative cells had increased expression of PROX1, transcription factor responsible for EEC differentiation. These changes in EECs and proliferative cells and increased basolateral secretion of secretory granules and serotonin was directly confirmed in colonoids (˃5 biologically unique human donors). The results of the current study demonstrate that uranium dust exposure directly induces transcriptomic changes in intestinal epithelia independently of microbiota, stroma, and immune cells. These results indicate that adult human colonoids are a relevant model for investigating environmental toxicants on intestinal epithelia and provide an improved understanding of uranium dust as a model of intestinal injury.

Emulating near-roadway exposure to traffic-related air pollution via real-time emissions from a major freeway tunnel system

KJ Bein, CD Wallis, JL Silverman, PJ Lein, and AS Wexler
University of California, Davis, CA, USA

Epidemiological and toxicological studies continue to demonstrate correlative and causal relationships between exposure to traffic-related air pollution and various metrics of adverse pulmonary, cardiovascular, and neurological health effects. The key challenge for in vivo studies is replicating real-world, near-roadway exposure dynamics in laboratory animal models that mimic true human exposures. The advantage of animal models is the accelerated timescales to show statistically significant physiological and/or behavioral response. This work describes a novel, state-of-the-art exposure facility adjacent to a major freeway tunnel system that provides a platform for real-time chronic inhalation exposure studies. Traffic-related air pollution is drawn directly from the tunnel system and delivered unaltered and in real-time to exposure chambers housed in an onsite animal vivarium. Results from over a year of continuous particle measurements during studies conducted at this facility will be presented with a focus on characterizing exposure dynamics. The primary conclusion is that particulate matter (PM) concentrations at this facility are routinely below the National Ambient Air Quality Standards (NAAQS), but studies completed to date still demonstrate significant neurological and cardiovascular effects. Internal combustion engines produce large numbers of ultrafine particles that contribute negligible mass to the atmosphere relative to NAAQS regulated PM2.5 but have high surface area and mobility in the body. It is posited here that current federal and state air quality standards are thus insufficient to fully protect human health, most notably the developing and aging brain, due to regulatory gaps for ultrafine particles; supported by the NIH (grants R21 ES025570, R21 ES026515, R01 ES026670, P30 ES023513, P30 AG010129, and RF1 AG074709).

Endotoxin exacerbates the NLRP3-dependent inflammatory potency of Saharan dust

Gerrit Bredeck[1], Jochen Dobner[1], Mathias Busch[1], Andrea Rossi[1], Burkhard Stahlmecke[2], Khanneh Wadinga Fomba[3], Hartmut Herrmann[3], Roel P. F. Schins[1]
[1]*IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany;* [2]*Institute for Energy and Environmental Technology e.V. (IUTA), Duisburg, Germany;* [3]*Atmospheric Chemistry Department (ACD), Leibniz-Institute for Tropospheric Research (TROPOS), Leipzig, Germany*

Epidemiological studies have shown that desert dust exposure affects respiratory health. About half of global desert dust is attributable to Saharan dust (SD). We aimed to investigate the oxidative and NLRP3 inflammasome-caspase-1-pathway-dependent inflammatory potency of SD compared to DQ12 quartz in submerged mono-cultures and more realistic air-liquid interface (ALI) co-cultures modeling the alveolar epithelium and macrophages.

Under submerged conditions, A549 cells as well as wild-type (WT) and NLRP3-/- THP-1 cells were exposed to SD and DQ12 at a concentration of 50 µg/cm². Using a Vitrocell Cloud12α, A549/THP-1 WT ALI co-cultures were exposed to SD and DQ12 at non-cytotoxic concentrations of 10, 20, and 30 µg/cm². Additionally, ALI co-cultures containing NLRP3-/- and CASPASE1/- THP-1 cells were exposed to SD.

SD contained endotoxin that could be inactivated by baking at 220°C. In A549 cells, SD but not DQ12 induced the expression of the oxidative stress marker gene heme oxygenase1. Conversely, only DQ12 upregulated the expression and secretion of interleukin (IL)-8. In WT THP-1 cells, SD and DQ12 caused manifold higher IL-1β secretions than in NLRP3/- cells. Endotoxin-free baked SD

induced IL-1β secretion to a ˜4-fold lower extent. Spiking baked SD with endotoxin partly restored the IL-1β secretion. In ALI co-cultures SD but not DQ12 upregulated the expression and secretion of IL-1β, IL-6, IL-8, and tumor necrosis factor α. The secretion of these four cytokines was strongly decreased in co-cultures with CASPASE-1/- or NLRP3/- THP-1 cells. Furthermore, preliminary data from long-read RNA sequencing indicated cytokine-mediated signaling pathways as the most activated gene set for SD exposure and agreed with the relatively low activity of DQ12.
The contrasting effects of SD and DQ12 suggested that, beyond NLRP3, distinct mechanisms drive their inflammatory effects. This is likely due to endotoxin and further microbial components in SD. The surprisingly strong SD-mediated activation of the NLRP3 inflammasome warrants further research on this common air pollutant.
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The Importance of a Multidisciplinary Approach in Inhalation Toxicology

Danielle Carlin, PhD, DABT
National Institute of Environmental Health Sciences

The National Institute of Environmental Health Science (NIEHS) and the NIEHS Superfund Research Program (SRP) fund research projects to address complex environmental health problems related to hazardous substances found at Superfund and other hazardous waste sites. Through a variety of funding mechanisms, the NIEHS and SRP support research in inhalation toxicology studies, which have included air pollution studies and nanomaterials to identifying therapeutic targets for preventing toxicity and understanding how chemicals may cause lung toxicity. The SRP uniquely emphasizes a multidisciplinary approach that combines biomedical, environmental science, and engineering research with community engagement, research translation, data science, and training efforts. Several SRP grantees study how exposure to harmful chemicals through inhalation can pose potential risks to human health. This includes aiming to understand the impacts of airborne contaminants — such as manganese, cadmium, arsenic, volatile organic compounds, polycyclic aromatic compounds — on cardiovascular and respiratory health. Specific examples include a predominantly African American community living near a Superfund site and metals in wind-blown dust near abandoned mines on Native American lands, both linking exposure to disease susceptibility. Scientists are also studying a class of recently recognized air pollutants, called environmentally persistent free radicals, and their long-term effects on lung and heart function. Responding to community concerns, SRP-funded researchers have studied asbestos and its potential harmful health effects, such as fibrosis and mesothelioma. SRP grantees also conduct engineering projects to remediate sites contaminated with hazardous substances and prevent their transport by air, such as by using plants, bacteria, and fungi to stabilize mine tailings and by developing carbon fiber-derived materials for air filtration in households. Others are carefully tracking airborne particulates, for example using predictive computational models, leveraging advances in nanotechnology, using plants as monitors, and documenting sources of air pollutants. Looking to the future, the NIEHS and the SRP will continue to support new research and technologies to address emerging challenges that may increase the harmful effects of inhaling environmental contaminants, such as complexities associated with non-chemical stressors, mixtures, and climate change.

Are ultrafine particles from aircraft of more concern compared to those from road traffic?

Flemming R. Cassee
Netherlands National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands; Institute for Risk Assessment Studies Utrecht University, Utrecht, the Netherlands

The exposure to ultrafine particles (UFP) in the vicinity of major airportsSchiphol can have an immediate effect on health according to research carried out by RIVM. Acute effects of short-term increases in the UFP (several hours to a few days) and the effects of long-term exposure (5 years) to ultrafine particles from air traffic. on health were investigated. Short-term reductions in lung function were measured in children and healthy adults as a result of higher short-term exposure. In healthy adults, also short-term reductions in heart function were measured. In addition lung cell cultures were exposed to collected UFP. On days with high exposures, children suffer more from respiratory complaints such as shortness of breath and wheezing. Children also use more medication on such days. These problems primarily affect children who already suffer from respiratory symptoms and already take medication in that regard. Toxicological analyses using in vitro lung models exposed to airport and non-airport (road traffic) UFPs as well as UFPs samples from a turbine engine revealed similar toxic properties.
Six types of health effects have been studied in the long-term exposure stduies: effects on the respiratory system (respiratory effects), effects on the cardiovascular system (cardiovascular effects), effects on metabolism (metabolic effects, including obesity), birth outcomes, neurological effects (effects on nervous system and psychological health), and general health (including all-cause mortality). Exposure to ultrafine particles from aircraft around Schiphol ('indicative evidence') could potentially lead to adverse effects on the cardiovascular system and the development of the unborn child. There is no evidence that long-term exposure to ultrafine particles is the cause of respiratory diseases. Existing conditions can be temporarily aggravated by short exposure. There is still insufficient evidence of effects on the nervous system and metabolism (diabetes). There are no indications for effects on total mortality.

Biography: Flemming Cassee, inhalation toxicologist since 1995, supports government authorities (national,WHO, EU) by coordinating and conducting research and providing advice to policy makers an regulators. His research focus is on aimbient particulate matter and airborne nanomaterials and microplastics. He is a professor in Inhalation Toxicology at the Institute for Risk Assessment Sciences at the Utrecht University, the Netherlands. He is also chief science officier at the National Institute for Public Health and the Environment (RIVM) of the Netherlands and Editor-in-Chief of Particle and Fibre Toxicology.

Who put gases (aldehydes) in my particles? Electronic cigarette-derived particles (and gases) in cardiovascular and pulmonary toxicity

Daniel J. Conklin, Ph.D.
American Heart Association – Tobacco Regulation and Addiction Center, Diabetes and Obesity Center; Christina Lee Brown Environmental Institute; Division of Environmental Medicine, Department of Medicine, University of Louisville, Louisville, KY 40292

Electronic cigarettes (e-cigarette) remain a popular electronic nicotine delivery system (ENDS) despite the recent banning of JUUL and fruity-flavored e-liquids. Moreover, health concerns regarding exposures to e-cig-derived aerosols remain given the devastating consequences of the EVALI outbreak (2019-2020) and the uncertainty of estimating disease risk of chronic use of e-cigs. As our research has implicated short-chain aldehydes as both biomarkers of exposure and as mediators of cardiovascular harm of tobacco products including e-cigarettes, we asked whether particles may be vehicles for aldehyde delivery into the lungs, and thus, particles contribute to cardiovascular and pulmonary effects. To address this broad question, we performed three experiments: 1) e-cig particles were characterized by size (mini-Moudi-based MMAD, GSD) and chemical composition (nicotine content; carbonyls by QDA trap) by UPLC-MS; and metal content by ICP-MS; 2) isolated murine aorta and human aortic endothelial cells were exposed to particle fractions and toxicity measured; and, 3) the acute cardiopulmonary effects of exposure of mice to e-cig aerosols (filtered or not) were recorded using radiotelemetry. Results of these studies show that semi-volatile/volatile gases partition into the particle phase; particles have direct toxicity on cardiovascular targets; and, acute cardiopulmonary responses are sensitive to e-cig-derived aerosols with limited effect of filtering particles. Additional studies are required to better understand the contributions of particle and gas phases of e-cig-derived aerosols to acute and chronic cardiovascular and pulmonary disease risk.

Repeated Exposure to Wildfire Smoke Alters Pulmonary Gene and Metabolic Profilesin Male Long-Evans Rats

Katelyn Dunigan-Russell[1], Janice A. Dye[2],Helen Nguyen[2], Hongzu Ren[2], Mette C. Schladweiler[2], Dean P. Jones[3], Anna Fisher[2], Ian M. Gilmour[2], Colette N. Miller[2],M. Ryan Smith[3], Kymberly M. Gowdy[1]
[1]Ohio State University; [2]U.S. Environmental Protection Agency; [3]Emory University

With the increasing prevalence and intensity of wildland fires in the U.S., there is significant concern for respiratory heath in occupational and public health settings. Wildfires increase toxins in the air including particulate matter (PM2.5), noxious gases, and volatile organic chemicals, increase lung inflammation and injury. We hypothesize that wildfire smoke inhalation induces pulmonary genetic and metabolicadaptations through inflammatory signaling. To test this, adult male Long-Evans rats were exposed to filtered air(FA)or smoke from eucalyptus biomass burning under smoldering conditions at a particle(32nm -10.57µm)concentration of 11 ± 1.89 mg/m3(low smoke, LS) or 23.7 ± 0.77 mg/m3(high smoke, HS) for 1 hr/day for 2 weeks. 24hrs following the last exposure, rats were euthanized and bronchoalveolar lavage (BAL) fluid and lung tissue were collected. BAL was used to measure differential cell counts, and cyto/chemokine production in the airspace. Lung tissues were assessed for changes in gene expression using RNA-seq paired with high-resolution metabolomics (HRM)(n=7-8/group). Differentially expressed genes (DEGs) were identified and analyzed by Ingenuity Pathway Analysis(IPA).BAL cell differentials revealed a dose dependent increase inmacrophages, and neutrophils following smoke exposure. Interferon-gamma(IFN-γ), and interleukin(IL)-10, IL-5 were also increased in the airspace following smoke inhalation. RNA-seq revealed 1,712 upregulated DEGs and 1,413 downregulated DEGs in the HS compared to FA. IPA analyses showed increased EIF2 and Rho Family GTPases. HRM revealed perturbations to 23 metabolic pathways including amino and fatty acids, antioxidant pathways with smoke exposure. Integrative analysis of the transcriptome and metabolome data revealed alterations in Wnt, NOS1,and Cox2. Integrative analysis and MWAS identified metabolic pathways and genes altered by HS, aligned with metabolic pathways and genes that are altered human Acute Respiratory Distress Syndrome. These results provide insights into the pulmonary response to wildfire smoke and support the association between wild land fire smoke exposure and adverse respiratory outcomes. All experiments were performed in accordance with the Animal Welfare Act and the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. This abstract does not reflect U.S. EPA policy.

Systemic Translocation of Polystyrene Microspheres: Quantitation Following Chronic Oral Gastric Exposure

Marcus A. Garcia[1], Seth D. Merkley[2], Eliane Hayek[1], Guy Herbert[1], Selita Lucas[1], Matthew J. Campen[1], Eliseo F. Castillo[2]
[1]University of New Mexico, Department of Pharmaceutical Sciences; [2]University of New Mexico, School of Medicine, Division of Gastroenterology and Hepatology, Department of Internal Medicine

Microplastics pollution and ingestion is an emerging environmental issue with uncertain impacts for human health.Global plastic use has consistently increased over the past century, and today many different types of plastics exist and are incorporated into every aspect of modern life. This has led to a substantial accumulation of plastics in the environment that slowly degrade into microplastics (MPs) that ultimately can enter the ecosystem, inhaled or ingested by both animals and humans alike, potentially leading to toxicity and adverse health outcomes. A major barrier for research in this field is the quantitation of systemic uptake and distribution of ingested or inhaled MPs. This study focuses on the impacts of MPs and establishes how they can pass through the gut barrier, leading to widespread accumulation in other tissues and organs such as the kidneys, liver, and lungs. Mice were exposed twice a week (2mg total) with 5µm polystyrene MPs via oral gastric gavage over a four-week period. After four weeks, the mice were euthanized, and lungs, liver, kidney, and cecum were extracted for evaluation of MP accumulation. Tissue digestion of liver and kidney was then performed by treating samples with 3x the tissue volume using 10% KOH. These samples were incubated at 40oC for 72 hours with agitation and then were ultracentrifuged for 4 hours at 30,000g. The supernatant was removed, and samples were re-suspended in EtOH and stored for imaging. Analysis of samples performed using the NanoLive 3D Cell Explorer microscope revealed significant translocation of polystyrene MPs into the liver by crossing the gut barrier. Polystyrene microspheres were also present in low concentrations within the kidney, which presents evidence of MPs systemic exposure via arterial circulation. Full quantitation of plastics concentration in peripheral tissues is further enabled by dissolution in nonpolar solvents and gas chromatographic assessment. MPs are unavoidable due to universal exposure, and the evolution of disease-associated is still largely unexplored. This study is focused on developing techniques to identify, isolate, and quantify MPs to aid in the early identification of poor health outcomes associated with MP exposure.

Comparative chemistry and toxicity of combustion emissions from biomass and synthetic materials

Dr M Ian Gilmour
US Environmental Protection Agency, Research Triangle Park, North Carolina USA

Exposure to smoke from combustion of biomass (oak, eucalyptus, pine, peat) or synthetic materials (plastic, plywood cardboard) is associated with health effects including reduced respiratory function and increased pulmonary inflammation, although differences attributable to fuel types and combustion conditions, and their subsequent chemistries have not been well defined. We have developed a laboratory based furnace system that can combust different fuels or other flammable substrates under flaming or smoldering conditions with subsequent inhalation delivery to mice. Smoke emissions are extensively analyzed for a suite of chemicals and health effects are monitored following a one hour exposure to either the whole or filtered smoke. Pulmonary function is measured before, during and after inhalation exposure and numerous markers of pulmonary injury and inflammation are assessed either 4 or 24 hours after exposure. Condensates from these combustion emissions are also tested for mutagenic potential in the "Ames assay" and the potency compared either by relative mass of condensate or by calculated emission factor. We found that of the biomass fuels, eucalyptus and peat had more potent effects on pulmonary function and inflammation that the other fuels (oak and pine) and these effects were amplified with exposure to flaming smoke. Plastic had the most potent effects of the synthetic materials combusted and again flaming conditions presented the highest effects. Filtration in general ameliorated the health impact (lung function and inflammatory biomarkers) but to different degrees depending on the fuel. The mutagenicity results mirrored the pulmonary phenotypes with flaming plastic being the most potent and flaming biomass also having greater effects than smoldering smoke, and these effects were in general associated with increased levels of polyaromatic hydrocarbons. Finally, data from these studies are being applied in non-targeted clustering algorithms to understand how different chemical signatures or classes are associated with distinct phenotypic endpoints. This approach provides a good platform for pulmonary toxicity testing of different combustion emissions and endpoints are being compared to high throughput testing of cell based systems in the hope that this predictive toxicity testing may be validated and replaced with non-animal testing systems. (This abstract does not represent U.S. EPA policy;)

Interactive outcomes after ultrafine particle and ozone mixed inhalation exposures

Salik Hussain DVM, PhD
[1]Department of Physiology and Pharmacology, School of Medicine, West Virginia University, Morgantown, USA

Environmental inhalation exposures are inherently mixed (gases and particles), yet regulations are still based on single toxicant exposures. While the impacts of individual components of environmental pollution have received substantial attention, the impact of inhalation co-exposures is poorly understood. We have recently developed and validated inhalation co-exposure system for ultrafine carbon black (CB) and ozone (O3) exposure. This talk will cover detailed characterization of co-exposure aerosols and their cellular and acellular reactivity. Characterization of ozone aggravated oxidant generation abilities of carbon particles using electron paramagnetic resonance (EPR) will be presented. We have identified a novel pathways of co-exposure induced lung function decline that involve oxidant mediated significant induction of epithelial alarmin (thymic stromal lymphopoietin-TSLP)-dependent interleukin-13 pathway. In addition, CB and O3 co-exposure cause unique transcriptomic changes in the lungs that are characterized by functional deficits to mitochondrial bioenergetics. In addition, novel findings on co-exposure induced altered lung remodeling in an acute lung injury model will be presented. Finally, findings on the contribution of Nod like receptor X1 (NLRX1) in environmental exposure induced lung inflammation and injury will be presented. In sum, evidence of interactive outcomes after air pollution constituent co-exposure and key mechanistic pathways after ultrafine carbon black and ozone inhalation co-exposure will be presented in this talk.

Pre-existent stress increases susceptibility to ozone and wildfire smoke exposure

TW Jackson[a], AR Henriquez[a], SJ Snow[b], DI Alewel[a], MC Schladweiler[b], KMP Jackson[c], CN Miller[b], PA Evansky[b], D Davis[b], M Monsees[a], AA Fisher[b], SA Vance[a], JA Dye[b], SH Gavett[b], UP Kodavanti[b]
[a]ORISE, EPA, RTP, NC; [b]PHITD, CPHEA, CIB, EPA, RTP, NC; [c]Metabolon Inc, Durham, NC

Current climate scenarios predict more frequent and longer-lasting heatwaves alongside rise in ambient ozone and particulate levels from widespread wildfires. These conditions are predicted to exacerbate mental health crises and chronic metabolic and immune disorders, especially in vulnerable individuals. We hypothesized that male Wistar-Kyoto (WKY) rats subjected to chronic variable stress, social isolation, or high temperature housing would be susceptible to metabolic effects from exposure to ozone or eucalyptus wildfire smoke. U.S. EPA Institutional Animal Care and Use Committee approved protocols prior to experiments. Initially, we exposed 5-week-old rats to variable stress paradigm or social isolation for 8 weeks and examined stressor interaction with subsequent ozone exposure. As previously demonstrated, ozone (0.8 ppm, 4 hours) increased urinary/plasma corticosterone/epinephrine and suppressed several pituitary and gonadal hormones. In addition, ozone increased markers of injury/inflammation (e.g. BALF markers, IL-6/Tnf-α). Interestingly, social isolation exacerbated ozone-induced effects and independently caused systemic inflammation that was exacerbated by ozone. Ozone increased glucose, cholesterol, branched chain amino acids, and social isolation exacerbated these effects. Metabolomic analysis revealed ozone-induced changes in lipid metabolism, whereas social isolation changed markers of sphingolipid metabolism linked with neurotransmitter functions and psychiatric disorders like depression. However, ozone-induced changes in selected metabolites of lipid processing were dampened in socially isolated animals when compared to pair-housed animals. In a follow-up study, male rats (4-week-old) housed at ~31 °C had a substantial reduction in body weight gain and increased lean mass compared to 22 °C housed rats. These animals, when exposed to wildfire smoke (~7 mg/m3 x 1hr), showed metabolic changes comparable to ozone-exposed animals (e.g. glucose intolerance). These data demonstrate that prior stresses increase susceptibility to neuroendocrine and metabolic effects of acute air pollution exposure, and that underlying stress likely exacerbates brain and systemic health conditions in individuals exposed to changing climatic conditions.

This abstract does not necessarily reflect US EPA policy.

Ultrafine particulate matter exposure aggravates pulmonary response to influenza infection during pregnancy

Nicholas L. Drury[1,5]; Toriq Mustapha[1]; Ross A. Shore[1]; Jiayun Zhao[2]; Gus A. Wright[3]; Aline Rodrigues Hoffmann[4]; Susanne U. Talcott[5]; Annette Regan[6]; Robert M. Tighe[7]; Renyi Zhang[2,8]; Natalie M. Johnson[1]

[1]Department of Environmental and Occupational Health, Texas A&M University, College Station, TX 77843; [2]Department of Chemistry, Texas A&M University, College Station, TX 77843; [3]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843; [4]Department of Comparative, Diagnostic, and Population Medicine, University of Florida, Gainesville, FL 32653; [5]Department of Nutrition, Texas A&M University, College Station, TX 77843; [6]School of Nursing and Health Professions, University of San Francisco, Orange County, CA 92868; [7]Department of Medicine, Duke University, Durham, NC 27710; [8]Department of Atmospheric Sciences, Texas A&M University, College Station, TX 77843.

Pregnant women are at high risk for severe morbidity and mortality from influenza infection. Although particulate matter (PM) air pollution exposure is known to modulate immunity and can impact pulmonary responses to viral infection, specific responses to ultrafine particles (UFPs, ⩽100 nm diameter), especially during pregnancy, are not well-characterized. To clarify the impact of UFPs on maternal respiratory health, we exposed time-mated C57Bl/6N dams to a PM mixture at a concentration of 100 μg/m3 (average particle diameter ˜70 nm) or filtered air (FA) from gestational days (GD) 0.5-13.5, followed by inoculation with a sub-lethal dose of Influenza A/Puerto Rico/8/1934 (PR8) or heat-inactivated (HI) control on GD14.5. Dams were evaluated for markers of disease severity 3 days post infection (dpi) prior to delivery. Dams infected with PR8 in both gestational exposure groups (PM and FA) exhibited significantly reduced weight gain. Pulmonary viral load was significantly higher in PM-exposed, PR8-infected dams (PM-PR8) in comparison to FA-exposed, PR8-infected dams (FA-PR8), as well as both HI control groups. Conversely, the FA-PR8 group showed the most extensive pathological findings on average, with moderate inflammation, which was less apparent in the PM-PR8 group due to early immunosuppression from PM exposure. Subsets of pulmonary T cell populations did not differ significantly between groups. Pulmonary expression of Sphk1 and Il-1β, encoding pro-viral and pro-inflammatory factors, respectively, were significantly increased in the PM-PR8 group, supporting a link between gestational UFP exposure and severity of influenza viral infection. Our findings demonstrate exposure to ultrafine PM during pregnancy aggravates pulmonary responses to influenza infection, characterized by increased viral titer, decreased cellular infiltrations early in infection, and increased expression of pro-viral and pro-inflammatory genes. Preventive measures, such as limiting UFP exposure and promoting vaccination, are warranted to protect maternal health.

Effect of Combustion Particle Morphology on Biological Responses in a Co-culture of Human Lung Epithelial (A549)and Macrophage-like (THP-1) Cells at Air-liquid Interface Exposure Conditions

Kamaljeet Kaur[1], Raziye Mohammadpour[2,4], Hamidreza Ghandehari[2,4,5], Christopher A. Reilly[2,6], Robert Paine III[3], and Kerry E. Kelly[1,2]

[1]Department of Chemical Engineering, University of Utah; [2]Utah Center for Nanomedicine, University of Utah; [3]Divisionof Pulmonary and Critical Care Medicine, University of Utah; [4]Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah; [5]Department of Biomedical Engineering, University of Utah; [6]Department of Pharmacology and Toxicology, Center for Human Toxicology, University of Utah

Combustion particles contribute significantly to the urban particulate matter and have been extensively studied for their associated health effects. The adverse health effects of combustion particles are well established, but the contribution from the specific physical (size and shape) or chemical properties remains uncertain. This study focuses on combustion particle morphology. The freshly produced combustion particles have a fractal-like morphology. However, the morphology of combustion particles changes from a fractal-like to more compact spherical-like shape during atmospheric aging. Previous studies of fresh and aged combustion particles have suggested that the changing chemical composition is the prime cause of the observed differences in toxicological response. However, little is known about the contribution of morphological changes in atmospherically aged particles to their toxicological response, possibly due to the difficulty in resolving the two properties (composition and morphology) that change simultaneously. A method to change particle morphology without the need to change chemical composition would help elucidate the effect of this morphological change on cellular responses. This study altered the shape of lab-generated combustion particles from fractal-like to a more compact spherical shape using water condensation and evaporation. Quantitative comparison of polycyclic aromatic hydrocarbons and semi-volatiles for both shapes (altered and not-altered) confirmed no significant change in composition. Using an electrostatic field-based air-liquid exposure (ALI) chamber, the two shapes were exposed to a co-culture of human airway epithelial (A549) and differentiated human monocyte (THP-1) cells. For the same mass dose of 2 mg/cm2, both shapes were ingested by cells, induced a pro-inflammatory response (IL-8 and TNFα) and enhanced CYP1A1 gene expression compared to air controls. The more compact spherical particles (representative of atmospherically aged combustion particles) induced more early apoptosis and release of TNFα compared to the more fractal-like particles. The result suggests a contribution of morphology to the increased toxicity of aged particles.

Cholesteroland particle-induced lysosomal membrane permeability

Kendall, Rebekah L., Holian, A

University of Montana

Exposure to micron-sized particles (<2.5µm) such as silica(cSiO2) and asbestos contribute to pulmonary and systemic diseases and present a critical global health issue. To develop more effective therapeutic strategies for these diseases, understanding the cellular mechanisms that contribute to particle-induced inflammation is crucial. Alveolar macrophages (AM) are the first line of defense against inhaled particles. Particles are taken into the macrophage via phagosomes that fuse with lysosomes to form phagolysosomes. cSiO2and other particles can permeabilize the phagolysosome and initiate the release of lysosomal enzymes that trigger the assembly and activation of the NLRP3 inflammasome and damage to other organelles. Thus, lysosomal membrane permeabilization (LMP) is the rate limiting step in particle-induced inflammation and disease. Cholesterol has been demonstrated to prevent LMP, but its role in particle-induced LMP and inflammation is not fully understood. We hypothesized that moderately increasing lysosomal cholesterol and lipid metabolism wouldprevent LMP in a particle-exposure model. Manipulation of

lysosomal cholesterol with the NPC2inhibitor U18666A protected against cSiO2-inducedIL-1βrelease. Additionally, inhibition of V-ATPase function to reduce lysosomal acidification was effective in reducing cSiO2-induced IL-1β. Cationic amphiphilic drugs (CAD) drugs can accumulate in lysosomes, disrupting acidification and causing cholesterol accumulation. Imipramine (IMP) prevented cSiO2-induced IL-1β release in vivo and in vitro and reduced fibrosis in mice.Hydroxychloroquine (HCQ) protected against cell death and inflammation in a similar manner. Fluvoxamine (FLV) and fluoxetine (FLX) also reduced cSiO2-induced LMP and IL-1βrelease in BMdM while reducing acid sphingomyelinase activity and cholesterol efflux. IMP, HCQ, FLV, and FLX each reduced lysosomal acidification and degradative function at early time points but largely recovered at 24 h, suggesting reversible effects on lysosomal pH and proteolytic function. Cholesterol accumulation was still evident after 24 h; total cholesterol increased similarly to U18666Atreatment. When cholesterol efflux was promoted in cSiO2 treated BMdM, IL-1βrelease increased significantly. A similar effect was observed with cellular cholesterol depletion. These findings suggest that modestly elevatedcholesterol can be protective of particle-induced LMP and inflammation, while diminished cholesterol enhances LMP and subsequent events.

Nanomaterial induced effects in the healthy vs. diseased liver

Ali Kermanizadeh[1,2], David Brown[2], Wolfgang Moritz[3], Vicki Stone[2]
[1]*University of Derby, School of Human Sciences, Derby UK;* [2]*Heriot Watt University, School of Engineering and Physical Sciences, Edinburgh, UK;* [3]*InSphero AG, Schlieren, Switzerland*

Hepatic toxicology is key following chemical, drug and nanomaterials (NMs) exposure, as the liver is vital in metabolic detoxification of chemicals as well as being a major site of xenobiotic accumulation. With ever-increasing production of NMs, there is a necessity to evaluate the probability of consequential adverse effects, not only in healthy but also in clinically asymptomatic liver, as part of risk stratification strategies. Here we present in vitro data generated in either healthy or diseased liver models mimicking steatosis and pre-fibrotic non-alcoholic steatohepatitis (NASH) constructed in a scaffold free 3D liver microtissue system composed of primary human hepatocytes, Kupffer cells, hepatic stellate cells and sinusoidal endothelial cells. Furthermore, the data generated in the in vitro models is compared and contrasted to in vivo data with same tested NMs. The highlights from the presentation will include: 1. Data demonstrating the establishment and validation of two in vitro disease models (steatosis and pre-fibrotic NASH) of the liver confirmed via histology, ATP content and triglyceride levels; 2. Strong experimental evidence for pre-existing liver disease being extremely important in the augmentation of NM-induced hepatotoxicity (in vivo - significant vast differences in histopathology, biomarkers of liver damage and inflammation and in vitro - magnitude in differences in NM induce cell death and inflammatory response between disease states); 3. Evidence for NMs activating stellate cells in the liver.
Overall, we suggest that it is imperative that all stages, or at least the clinically asymptomatic and benign forms of the wide-spectrum of liver disease are incorporated in risk assessment strategies. This is of significant consequence, as the majority of the general adult population suffer from sub-clinical liver injury without any apparent or diagnosed manifestations.

Understanding the Lung-Gut Axis by Modeling the Influence of Genetic Diversity, Welding Fume Inhalation Exposure, and Lifestyle, on the Profile of Gut Microbiome

Vamsi Kodali[1,2], Salik Hussain[2], Katherine A Roach[1], Michael Kashon[1], Theresa Boots[1], Mohammad Shoeb[1], Gregory Boyce[1], Terence Meighan[1], Tracy Eye[1], Jenny R Roberts[1], Patti C Zeidler-Erdely[1,2], James M Antonini[1], Aaron Erdely[1,2]
[1]*Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV;* [2]*School of Medicine, West Virginia University, Morgantown, WV*

The gut microbiome has a modifying influence on various systemic organs and dysbiosis in the microbiome correlates with various diseases and pathological conditions. The goal of the current work was to profile the influence of genetic diversity (inbred vs. outbred), occupational pulmonary exposure (welding fume), and lifestyle (diet) on the gut microbiome. Outbred (Sprague-Dawley, SD) and inbred (Brown Norway, BN) rats were maintained on a regular chow (RG) or high fat (45 kcal % fat, sucrose 22.2 % by weight, HF) diet for 24 wk. At wk 7, groups of rats maintained on each diet were exposed by inhalation to stainless steel welding fume (WF; 20 mg/m3 x 3 h/d x 4 d/wk x 5 wk) or filtered air until wk 12, at which time some animals from each group were euthanized. A separate set of rats from each group were allowed to recover from WF exposure until wk 24. The DNA from the feces was extracted and sequenced for bacterial 16s. In terms of the exposure, WF induced pulmonary injury and inflammation in both strains and diet had minimal influence. Resolution of inflammation was observed for the SD strain but not the BN strain. WF inhalation caused a significant change in gut alpha diversity in RG-diet fed rats. As expected, HF diet altered gut alpha diversity compared to RG diet-fed rats, which was not influenced by WF exposure. Richness, a measure of number of species in the gut microbiome, was altered with HF diet and further altered by WF exposure. Exposure-specific microbiome kinetics were identified. Specific bacterial populations were altered by WF, HF diet, and the combination of the two (e.g., Verrucomicrobia and Actinobacteria). Bacterial population changes correlated with various pulmonary and systemic toxicity endpoints. Proportion of variation analysis indicated alteration in the microbiome after exposure and recovery was influenced the most by genetic variation followed by diet and pulmonary exposure. The results suggest that the dysbiosis in the gut microbiome caused by lifestyle can be exacerbated by inflammatory insults like pulmonary exposures as well as genetic diversity.

The effects of chronic exposure to ambient traffic-related air pollution on Alzheimer's disease phenotypes in wildtype and genetically predisposed male and female rats

PJ Lein, KT Patten, AE Valenzuela, C Wallis, KJ Bein, AS Wexler
University of California, Davis, CA, USA

Epidemiological studies consistently link traffic-related air pollution (TRAP) to increased risk of Alzheimer's disease (AD). Preclinical data corroborating this association are largely from studies of male animals exposed acutely or subchronically to high levels of isolated fractions of TRAP. What remains unclear is whether chronic exposure to ambient TRAP modifies AD risk and the influence of sex on this interaction. To address these gaps, male and female TgF344-AD rats (Tg) that express human AD risk

genes and wildtype (WT) littermates were housed in a vivarium adjacent to a heavily trafficked tunnel in Northern California and exposed for up to 14 months to filtered air (FA) or TRAP drawn from the tunnel and delivered to animals unchanged in real-time. Particulate matter (PM) concentrations in TRAP exposure chambers fluctuated with traffic flow but remained below 24-hour PM2.5 NAAQS limits. Ultrafine PM was a predominant component of TRAP, and nano-sized refractive particles were detected in the hippocampus of TRAP males and females by hyperspectral imaging. TRAP accelerated amyloid proteinopathy in Tg males and females, increased phosphorylated tau in WT males, promoted neuronal cell loss in both genotypes and sexes, and caused cognitive deficits in WT males. TRAP had no effect on astrogliosis, but modulated microglial cell activation in Tg and WT males and females, although the temporal profile varied between sexes. The results of this realistic, chronic, and low-concentration exposure suggest that ambient TRAP promotes the progression of AD via complicated interactions with age, sex, and genotype. These findings suggest current PM2.5 regulations are insufficient to protect the aging brain. All animal studies were conducted humanely and in accordance with protocols approved by the University of California, Davis Institutional Animal Care and Use Committee. Supported by the NIEHS (grants R21 ES025570, P30 ES023513 and T32 ES007059) and NIA (grant P30AG010129).

Sputum macrophage carbon load as a biomarker for episodic exposure to ambient combustion particles

Menghui Jiang[1] , Huining Kang[1] , Cassie Rowe[1] , Jialiang Wang[1] , Chris Dagucon[2], Yan Lin1, Akshay Sood[1] , Xiaozhong Yu[1] , Steven Belinsky[2], Shuguang Leng[1]
[1] *University of New Mexico;* [2]*Lovelace Biomedical Research Institute*

Combustion-emitted particulate matter (CE-PM) has become a top-priority issue of public health and climate change in the United States (US) and globally. CE-PM from traffic, industry, wildfire, and wood burning is the major contributor to ambient and indoor air PM2.5 pollution in the US. These major CE-PM emissions have different temporality and spatiality, and quantification of inhalation exposures from the total environment at the individual level challenged all existing environmental assessment approaches. Macrophage carbon load (MaCL) is a novel sputum cytology-based method that quantifies black carbon particles engulfed by macrophages and reflects lung dose of total CE-PM exposure at an individual level over the past several months. We selected archived sputum slides from 69 subjects enrolled in the Lovelace Smokers cohort (LSC) based on various PM2.5 levels in weeks prior to sputum collection. Western IRB approved this study and all participants signed consent form. Annual PM2.5 levels in Albuquerque and surrounding areas where the LSC enrollment targeted were about 6 µg/m3 and extended periods (e.g., >14days) with higher PM2.5 (e.g., >10 µg/m3) indicate wildfire smoke invasion due to wild fires in surrounding counties or states. Using an Olympus BX43 microscope equipped with a motorized Z-drive, we obtained stack images with 100 nm depth interval for each field and generated projection images. Size of macrophages (n=50 per slides) and number and area of the engulfed particles were manually analyzed using NIH ImageJ. Major findings include: 1) median diameter of engulfed particles is 0.35 µm with 90% engulfed particles having diameters <1.31 µm; 2) MaCL levels quantified as median number or areas of particles correlate well with PM2.5 levels in the past several weeks but not months; 3) subjects with self-reported ever wood smoke exposure have higher MaCL levels equivalent to changes caused by exposure to 10 µg/m3 PM2.5; and 4) smoking status and packyears were not significantly associated with MaCL levels. These findings support MaCL assay as a biomarker for episodic exposure to ambient combustion particles in the past several weeks. Currently we are applying a neural network based scoring algorithm to analyze the images and compare the results with manual counting.
This study was supported by NCI grant P30 CA118100 and NIGMS U54 GM104944.

Characterization and in-vitro toxicology of Subway PM

David Luglio, John Adragna, & Terry Gordon
New York University School of Medicine, New York, New York 10010

Subways around the world are known to have very poor air quality, with PM2.5 concentrations several times ambient street-side levels. These particles are predominantly iron with notable amounts of other heavy metals. We show this is the same case on underground subway platforms of Boston, Washington, DC, and the New York City metropolitan area in the Northeastern United States. In fact, one transit system, the Port Authority Trans-Hudson (PATH), had some of the highest PM2.5 concentrations ever recorded (i.e., average of 779 +249 µg/m3). Detailed mineralogical and oxidative state analysis of showed that the iron in subway aerosols is a mix of Fe(II) and Fe(III), and found in the forms of hematite (Fe2O3), magnetite (Fe3O4), ferrihydrite (Fe3+10O12(OH)2), goethite (FeO(OH)), and wüsite (FeO). Ferrihydrite and goethite composed, on average, 51% of the iron particles and are indicative of rust, whereas hematite and magnetite compose, on average, 40% of the iron particles and are produced in high energy processes. The toxicology of these particles was investigated using Calu-3 lung epithelial cells in submerged media. PM concentrations of up to 100 µg/ml did not increase cell death, but concentrations as low as 50 µg/ml caused the production of oxidative stress. Induction of oxidative stress in cells was found to be dependent on the station where the PM was collected. Furthermore, comet assay results have indicated that more DNA damage occurs in treated cells than in a negative control. The alarmingly high concentrations of PM2.5 in subway stations in the Northeastern United States clearly indicate the need for air quality remediation. Our in vitro toxicological results add further credence to the fact that commuting on subways could be harmful to a person's health. With knowledge of the composition of subway PM, we can better understand the mechanisms of toxicity as well as better target the sources of the pollution. Future directions will target human exposure studies to evaluate the potential for adverse health effects in individuals present for extended times on a subway platform or train.

Health Risks of Emissions of Internal Combustion Engines: A Success Story Joining Science, Technological Developments and Policy

Roger O. McClellan
Independent Advisor, Toxicology and Risk Analysis
Albuquerque, NM, 87111

Spark ignition and compression ignition engines, the latter commonly referred to as Diesel engines, were invented in the late 1800s and quickly became a cornerstone of the industrial revolution. Typically, both are fueled by petroleum fuels. The

combustion process yields energy and a mixture of combustion products including carbon dioxide (CO2), carbon monoxide (CO), nitrogen oxides (NOx), carbon particles and hydrocarbons. These engines soon found wide application leading to advances in engine and fuel technology. An early concern for the gasoline engines was emission of carbon monoxide and for Diesel engines "black smoke". Later concern developed for "smog" produced by photochemical enhanced reactions between oxides of NOx and hydrocarbon compounds. Exhaust control systems were soon developed that required removal of Pb from the fuel. Improvements in engine technology, fuels and emission controls markedly reduced the CO, NOx and hydrocarbon emissions from gasoline spark ignition engines. In 1955 it was shown that organic extracts of exhaust painted on mouse skin caused cancer. In 1977, it was shown that Diesel exhaust particles contained an array of complex hydrocarbons that were mutagenic. These findings lead to concern for engine emissions causing cancer. In the 1980s it was shown that chronic inhalation exposure to high concentrations of Diesel exhaust caused lung cancer in rats. Early epidemiological studies yielded equivocal results for cancer induction. The International Agency for Research on Cancer (IARC) in 1988 categorized gasoline engine exhaust as "possibly carcinogenic to humans" and Diesel exhaust as "probably carcinogenic to humans". Further action was taken to remove the hydrocarbon from Diesel exhaust. In 2012 IARC elevated its classification of exposure to Diesel to "carcinogenic to humans" without distinguishing between emissions from traditional and new technology engines. Nonetheless, this is a remarkable success story of using science to inform both technological advances and regulatory policies. Recently, Societal concerns have shifted to "climate change" driven by concern for "greenhouse" gas emissions, including CO2 dioxide, from internal combustion engines. This presentation will provide possible options for the path forward.

Exposure to eucalyptus smoke during sperm maturation alters motility and non-coding RNAs in caudal sperm

Colette N. Miller[1] , Janice A. Dye[1] , Lillian F. Strader[1] , Gail M. Nelson[2], Mette C. Schladweiler[1] , Helen Nguyen3, Gleta K. Carswell[2], Grant Palmer[3], Ian M. Gilmour[1] , Brian N. Chorley[2], Gary R. Klinefelter[1]

[1] Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, U.S. EPA; [2]Biomolecular and Computational Toxicology Divison, Center for Computational Toxicology and Exposure, U.S. EPA; [3]Oak Ridge Institute for Science and Education, U.S. EPA

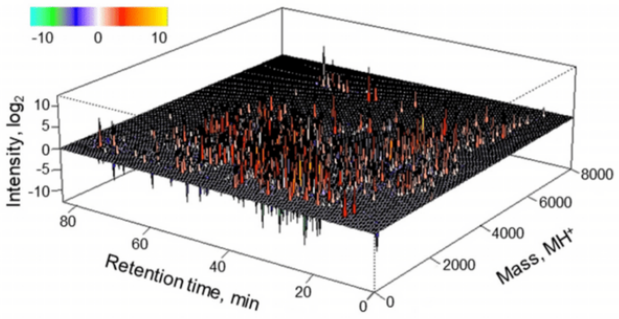
Concern about infertility in firefighters has been raised since the early 1990s, in part due to variable occupational exposures that span from heat stress to the thousands of chemicals in smoke. While hyperthermia is a well-recognized male reproductive hazard, little information exists on the effects of inhaled biomass smoke on fertility. Hence, the purpose of this study was to determine if exposure to smoke from eucalyptus combustion induces adverse effects on sperm. Long-Evans rats were exposed for1 hour to either a low or high concentration of smoke generated bya tube furnace system set to 500°C for 4 consecutive days on the first week and 3 days on the second week. Daily mean particulate matter and CO concentrations were 11.0 mg/m3and 11.4 ppm, or 23.7 mg/m3and 20.9 ppm for the low and high exposures, respectively. Caudal sperm samples were collected for functional and transcriptomic alterations the day after the final exposure. Sperm collected from the low exposure group had a 5% reduction in progressive tracking compared to rats exposed to filtered air, indicating an impairment for linear movement. Notably, this decrease was accompanied by alterations in the non-coding RNA populations found in mature sperm by Illumina RNA seq. A total of 14 microRNAs, including ones already associated with reproductive disorders, were increased in the low exposure group (e.g., miR-10b, -146, and -92). An additional 79 tRNA-derived fragments and5 piwi-interacting RNAs were different between the low exposure and filtered air groups. Importantly, these changes in sperm progressive movement and non-coding RNAs were not found in the rats exposed to high smoke concentrations. We have recently replicated the effects of smoke on sperm motility at a lower concentration of ˜4 mg/m3, which persisted despite filtration. Collectively, our work in rats provides novel evidence that exposure to biomass smoke can have potential adverse effects on sperm motility and necessitates the continued study of this exposure on male fertility and the developmental outcomes in their offspring. This study was approved by the U.S. EPA IACUC and does not reflect U.S. EPA policy.

Serum Peptidome: Diagnostic Window into Pathogenic Processes following Occupational Exposure to Carbon Nanomaterials

Andrew K. Ottens

Department of Anatomy and Neurobiology, Virginia Commonwealth University

Growing industrial use of carbon nanotubes and nanofibers (CNT/F) warrants consideration of health outcomes. While epidemiology among CNT/F workers reports on few acute symptoms, there remains concern over sub-clinical CNT/F effects that may prime for chronic disease, necessitating sensitive health diagnostic markers for longitudinal follow-up. CNT/F produced pulmonary, cardiovascular, neurological and other toxic effects in animals. In modeled exposure, bioactive peptides were found released into the circulation, the augmented serum peptidome, largely as products of matrix proteases in the lung. In the periphery, shed peptides were found to acutely augment vascular function, including the cerebrovasculature where neuroinflammation extended well into the parenchyma and influenced synaptic balance. Longer-term, neuroinflammation remained evident and coincident to an increased accumulation of amyloid pathology, exhibiting a phenotype consistent with early neurodegenerative disease. Moving to human occupational exposure, the serum peptidome exhibited the capacity to discriminate those exposed to higher (>0.5 µg/m3) or lower (<0.1 µg/m3) levels of inhalable CNT/F within the industrial setting. A top-five peptide biomarker model offered ideal prediction with high accuracy (Q2=0.99916) and a strong linear correlation with personal CNT/F exposure. Identified peptides associated with vascular pathology. ARHGAP21, ADAM15 and PLPP3 peptides implicated heightened vascular permeability and F13A1, FBN1 and VWDE peptides inferred a pro-thrombotic state among those with higher CNT/F exposures. In conclusion, the serum peptidome affords a diagnostic window into pre-symptomatic pathology among CNT/F exposed workers that was consistent with vascular consequences observed in modeled exposures and offers utility in longitudinal monitoring



Augmented serum peptidome in workers with CNT/F exposure >0.5 µg/m³.

Bridging the Pulmonary Toxicity of Inhaled Gaseous, Liquid- and Solid-Aerosol Phases

Jüergen Pauluhn
Covestro, Global Phosgene Steering Group, Leverkusen, Gemany

Lipophilicity enhances the trapping and dissolution of inhaled substances in lung regions lined with amphiphilic fluids. The amphiphilic pulmonary surfactant (PS) lining the alveolar region provides a unique interface for trapping and dissolving inhaled substances. Its major constituent is phosphatidyl choline (PC) with a pool size of about 11 µmol PC/kg-body weight. The PC-flux/h is about one third of this pool size. Inhalation dose-rates exceeding this PC-pool size during a continuous exposure period may cause a dose-rate related acute depletion of functional PS by adsorptive or chemical processes. The ensuing imbalances of lungfluid dynamics are counteracted via Specialized Pro-resolving Mediator (SPM) pathways which commonly are perceived as 'substance-specific'; however, may be better described as high dose-rate related dynamic phenomena. A typical accompaniment are increases in the pool of total cells (TCC) caused by increased counts of neutrophils (PMN) and PC- and substance-laden alveolar macrophages (AM). As conceptualized in Fig. 1, the increase of PMN in bronchoalveolar lavage (BAL) parallels the increase in AM, suggesting that the overload-dependent pool of AM controls that of the PMN. Depending on the degree of phospholipidosis, the residence time of biodegradable PC in the AMs is short (t1/2 ≈2 weeks) or markedly longer (t1/2 ≈10-120 weeks) for poorly soluble particulates if chronically inhaled at lung overload conditions. The degree of substance- and dose-specific depletion of PS appears to be a unifying denominator of pulmonary toxicity. This hypothesis is challenged in rat inhalation studies with lipophilic, reactive phosgene gas, poorly soluble solid aerosols of cationic amphiphilic drugs (CADs), ZnO and Zn-chelate, anionic polyurethane polymer nano-dispersions (APUDs, molecular weight >20000; liquid/semi-solid with both lipophilic and hydrophilic moieties) and non-reactive Carbon Nanotubes (MWCNT). Although physicochemically and chemically different, all substances compared shared some interactions with PS at different degrees. It seems, as if PS is a modulating key player in the etiopathology in pulmonary toxicology for both gaseous, liquid, and solid phases. In summary, the modulating factors of PS on pulmonary outcomes must be understood to better differentiate high-dose phenomena from substance-specific adversities.

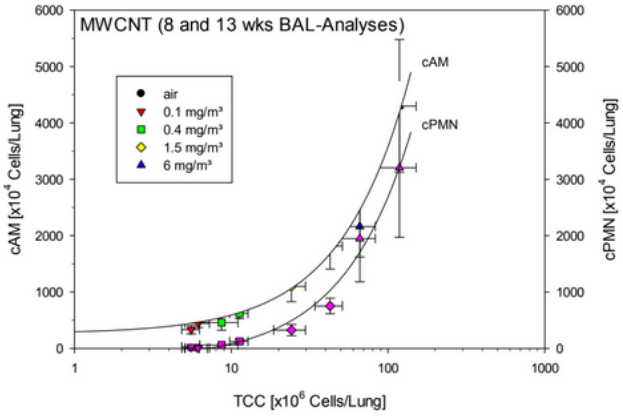


Figure 1: Interrelationship of the total cell counts (TCC) of cAMs and cPMNs of rats exposed to MWCNT for 8 and 13 weeks.

Inhalation toxicity of copper sulphate pentahydrate and dicopper oxide

Craig Poland[1,2], Sue Hubbard[3], Len Levy[4], Yamini Gopalapillai[5], and Carol Mackie[1]
[1] *Regulatory Compliance Limited, Loanhead, Edinburgh, UK;* [2]*Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK;* [3]*SAHCo Ltd, Chester CH3 7JW, UK*
[4] *Cranfield University, Cranfield, U;* [5]*Material Stewardship, International Copper Association, Washington, DC, USA*

Inhalation exposure to copper may occur in a variety of occupational environments ranging from the mining and refining of copper metal to the production and use of copper compounds. To investigate the potential inhalation effects of copper exposure, a 14-day range finding study was conducted using two copper compounds, differing in solubility and representative of copper substances in large-scale production/use. Crl:CD(SD) rats were repeatedly exposed to aerosols of dicopper oxide (Cu2O) or copper sulphate pentahydrate (CuSO4.5 H2O) at normalised copper doses of 0.18, 0.71, 1.78 and 8.9 mg/m3 Cu. There was a dose-related increase in lung macrophages with an acute influx of polymorphonuclear leukocytes (neutrophils) at ³1.78 mg/m3 Cu for both compounds whilst only CuSO4.5 H2O exposure resulted in epithelial cell hyperplasia. This epithelial response may reflect the rapid dissolution of CuSO4.5 H2O in lung lining fluid leading to a release of copper ions at the epithelial surface whilst Cu2O is relatively indissolvable at neutral pH.

The inhalation effects were studied in more detail during a 28-day study with Cu2O at doses of 0.2, 0.4, 0.8 and 2.0 mg/m3 Cu2O following OECD TG 412. To assess the temporal response with ongoing exposure, satellite groups were exposed for 1-, 2- or 3- weeks in addition to a 13-week post-exposure recovery period group. The response was characterised by localised alveolar histiocytosis and neutrophil influx with no evidence of epithelial hyperplasia or fibrosis in the lung and no systemic effects. The satellite groups showed that this acute inflammatory response peaked between weeks 1-3 and reduced thereafter despite ongoing exposure. This biphasic response may indicate adaptation and a shift towards a pro-resolution response with all biomarkers returning to control levels during the post-exposure recovery period. Overall, these results show that repeated exposure to copper compounds results in a controlled acute cellular response with no associated pathology and which fully resolved after the cessation of exposure.

A Case for Amorphous Silica Exposure in the Development of Chronic Kidney Disease of an Unknown Etiology

Keegan L. Rogers[1] , Carlos A. Roncal[2], Richard J. Johnson[2], and Jared M. Brown[1]
[1] *Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Anschutz Medical Campus, Aurora, CO, 80045;* [2]*Division of Renal Diseases and Hypertension, School of Medicine, University of Colorado, Anschutz Medical Campus, Aurora, CO, 80045*

We have hypothesized that sugarcane burning in developing countries contributes to an endemic kidney pathology in agricultural workers called chronic kidney disease of an unknown etiology (CKDu). Elemental analysis shows that sugarcane stalks contain a high percentage of amorphous silica (SiO2). We hypothesized that burning of sugarcane generates and releases nano-sized silica particles into the environment. These silica nanoparticles (SiNPs) present an inhalation and ingestion exposure to these agricultural workers. To determine if SiNPs are present in environmental matrices and kidney biopsies from agricultural workers, we utilized single particle inductively coupled plasma mass spectrometry (ICP-MS). Using single particle ICP-MS, we identified SiNPs within digested sugarcane ash which ranged in size from 190-212 nm. In kidney tissue, we identified the same representative SiNP population in a diagnosed CKDu patient that was not present in a negative patient. In a small cohort of patients, we found a significant increase in the number of SiNPs in the kidney biopsies of patients with a likely diagnosis of CKDu who were individuals who either worked in agricultural fields or their primary source of drinking water was from a shallow well. To determine the cellular effects of SiNPs on the kidney, we used a human proximal convoluted tubule (PCT) cell line (HK-2) to recapitulate the nephron's exposure to sugarcane ash, silica-free ash, and sugarcane ash derived SiNPs at 0.25, 2.5, and 25 µg/mL. Despite not being directly cytotoxic to HK-2 cells at 24 hours at any concentration, SiNPs were taken up and generated reactive oxygen species within 24 hr of exposure at all doses. Vimentin staining confirmed HK-2 cells underwent epithelial-mesenchymal transition (EMT) following treatment with ash or SiNPs but not with silica-free ash. These findings suggest SiNPs present in sugarcane ash are capable of driving EMT in PCT cells potentially contributing to a CKDu phenotype.

A Panorama of Airborne Particles: From Then to Now and From Local to Global

Jonathan M. Samet MD, MS
Colorado School of Public Health

Airborne particles have long posed a global public health threat, causing epidemics of death during such notorious episodes as the London Fog of 1952 and contributing to an enormous burden of avoidable morbidity and mortality worldwide. Decades of research have deepened our understanding of how inhaled particulate matter (PM) causes injury and disease and brought new tools for investigating PM: small and accurate monitors for capturing personal exposure, models for estimating PM concentrations across the world, and in-vitro methods for investigating pathways of injury by particles. Over the decades since the mid-20th century, epidemiological research has causally linked exposure to PM indoors and outdoors to an ever-growing list of adverse health outcomes, extending from early life effects to premature mortality. The resulting burden of disease estimates are staggering, amounting to millions of lives lost prematurely. The epidemiological evidence in combination with deeper insights from toxicological and exposure sciences have been the basis for ever lower standards and guidelines for ambient PM concentrations. However, critical questions remain unanswered. Most significantly for supporting targeted control of the most critical PM sources, we have yet to have sufficient understanding of the PM characteristics that drive particle toxicity for the various adverse health effects of exposure and cannot link critical characteristics to sources. This presentation provides a historical perspective on the emergence of the evidence on PM and health, covering critical advances and their consequences. It then turns to the present, covering the profile of PM exposure globally and the associated burden of morbidity and premature mortality. It ends with a look to the future, covering research needs to provide evidence that will support further control of PM globally.

(This presentation does not involve animal and/or human research directly)

The effects of traffic-related air pollution exposure on Alzheimer's disease-like pathology in mice

R.P.F. Schins[1], T. Wahle[1], A. Sofranko[1], E. Scarcello[1], I. Masson[1], C. Albrecht[1], G. Bredeck[1], M. Busch[1], P. Fokkens[2], J. Boere[2], F. R. Cassee[2,3]
[1]*IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany;* [2]*National Institute for Public Health and the Environment, Bilthoven, the Netherlands;* [3]*Institute for Risk Assessment Sciences, Faculty of Science, Utrecht University, Utrecht, the Netherlands*

A growing number of epidemiological studies has linked exposure to traffic-related air pollution (TRAP) to neurological and neurodegenerative diseases. The air pollution in traffic-dominated urban environments is represented by high numbers of combustion-derived ultrafine particles that have been shown to translocate to the brain. However, this air pollutant mixture consists of various other constituents including abrasion-derived particles from brakes and tyres, volatile organic compounds like benzene and gaseous compounds like nitrogen oxides and ozone. In a previous study, we showed that subchronic inhalation exposure to diesel engine exhaust, as a model of TRAP, aggravates amyloid-β plaque formation and motor function impairment in the 5xFAD transgenic mouse model of Alzheimer's disease (AD). To further strengthen the epidemiological association between TRAP and AD we exposed 5xFAD and wildtype littermate mice for 5 h/days, 5 days/week during 2 or 4 consecutive weeks at a representative urban traffic-dominated location to: (I) concentrated ambient particles, (II) particle filtered ambient air or (III) clean air (control). Experiments were in accordance with German Animal Welfare Legislation and performed as approved by the North Rhine-Westphalia Office of Nature, Environment and Consumer Protection (LANUV, NRW).
Our study revealed that repeated inhalation exposure to TRAP accelerates plaque formation in the 5xFAD mice. Furthermore, we found that the gaseous fraction of the air pollutant mixture probably plays only a minor role in this effect. The major outcome of this study substantiates the role of air pollution in AD and suggests that long-term exposure to traffic-related air pollution particles is a risk factor for this debilitating disease.
Acknowledgements: The work leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No

814978 (TUBE) and a cross-border grant awarded by the Alzheimer Forschung Initiative (AFI, Germany) and Alzheimer Nederland.

Neurometabolomic Impacts of Modeled Wildfire Smoke Exposure in Aged Female C57BL/6 Mice and Protective Benefits of Resveratrol, Nicotinamide Mononucleotide (NMN), and Senolytics

David Scieszka, Haiwei Gu, Russell P Hunter, Ed Barr, Marcus Garcia, Jessica Begay, Guy Herbert, Kiran Bhaskar, Mark McCormick, Rama Gullapalli, Rahul Kumar, Barry Bleske, Andrew Ottens, Matthew Campen

Department of Pharmaceutical Sciences, College of Pharmacy, University of New Mexico; Florida International University Center for Translational Sciences Departments of Neurosciences, Biochemistry, and Pathology, School of Medicine, University of New Mexico; Department of Neurosciences, School of Medicine, Virginia Commonwealth University

Wildland fires have become progressively more extensive over the past 30 years in the US, and now routinely generate smoke that deteriorates air quality for most of the country. We explored the impact that smoke derived from biomass has on the metabolomic profile of brains from older (18 months) female C57BL/6 mice acutely and after 10 weeks of recovery from exposures. Mice (N=6/group) were exposed 4 hours/day every-other-day for 2 weeks (8 exposures total) to concentrations of woodsmoke averaging 0.6 mg PM2.5/m3. One group of mice was euthanized 24 hours after the last exposure. Other groups were then placed on 1 of 4 treatment regimens: vehicle; resveratrol plus NMN (Resv+NMN), senolytics (dasatanib+quercetin; D+Q); or both Resv+NMN and D+Q. At sacrifice, brains were rapidly excised and frozen for untargeted metabolomics. Among the findings, the aging from 18 months to 21 months was associated with the greatest metabolic shift, while exposure effects were relatively modest. Of the drug regimens, none were able to offset the full effect of aging, but the combination of Resv+NMN with D+Q exhibited the greatest metabolic shift. Immediately after wildfire smoke exposure, modest neurometabolite changes were observed (6 metabolites upregulated, 10 downregulated), but after 10 weeks a persistent difference in exposed mice could be seen (5 up, 12 down, including significant reductions in serotonin). The wildfire smoke exposure effect on serotonin was not seen in groups treated with Resv+NMN or D+Q. The results are preliminary and will be presented in context with pulmonary and cardiac findings from these groups.

Lipid Mediated Exacerbation of Nanoparticle-Induced Pulmonary Inflammation

Saeed Alqahtani,[1] Christina Ferreira,[2] Li Xia,[1] and Jonathan Shannahan[1]

[1]*School of Health Sciences, College of Health and Human Sciences, Purdue University;* [2]*Purdue Metabolite Profiling Facility, Purdue University*

Prevalent underlying diseases, such as metabolic syndrome (MetS), enhance susceptibility to inhaled exposures. Lipids are dysregulated in many diseases and are intricately involved in inflammatory signaling facilitating both initiation and resolution. Our data demonstrate exacerbated acute as well as sustained pulmonary inflammation in a MetS mouse model compared to a healthy model after 20nm silver nanoparticle (NP) exposure. Exacerbated inflammation corresponded with inhibition of numerous lipid resolution mediators. Therefore, we hypothesized modulation of pulmonary lipids could be utilized as a treatment strategy to address the exacerbated pulmonary inflammation in MetS following inhaled particulate exposures. Healthy and MetS mice were treated by incorporation of atorvastatin into their diet, exposed to 20nm silver NPs (50ug), and evaluated for acute toxicity endpoints 24h post-exposure. Responses were compared to a cohort not receiving atorvastatin. Treatment with atorvastatin reduced exacerbations in neutrophilic influx and cytokine/chemokine levels in MetS following NP exposure to levels observed in exposed healthy mice. A lipid profiling approach determined atorvastatin inhibited alterations in lipid mediators of inflammatory resolution observed in MetS mice following NP exposure. These findings suggest lipid dysregulation may contribute to exacerbated toxicity associated with MetS. To examine the contribution of specific resolution mediators, a cohort of mice were treated with precursors of resolution mediators (14HDHA, 17HDHA, or 18HEPE) prior to NP exposure. Treatment with 14HDHA or 17HDHA prior to NP exposure reduced exacerbations in pulmonary neutrophilia in MetS mice while 18HEPE was ineffective. A targeted lipid assessment approach demonstrated treatments elevated distinct resolution mediators within the lung. Based on these data, healthy and MetS mice were exposed to NPs and treated with a distinct resolution mediator, RvD1, 1-day post-exposure. This treatment reduced pulmonary NP-induced inflammation. Overall, our evaluations suggest therapeutic targeting of lipids may benefit individuals suffering from diseases associated with lipid dysregulation following inhaled toxicant exposure. All animal procedures were approved by the Purdue University Animal Care and Use Committee. Funded by NIEHS R01ES033173.

Neurotoxic Responses to Diesel Exhaust Particle NIST 2975

Kristina Shkirkova[1], Krista Lamorie-Foote[1], Nathan Zhang[2], Andrew Li[2], Arnold Diaz[3], Qinghai Liu[1], Max A Thorwald[3], Jose A. Godoy-Lugo[3], Brandon Ge[1], Carla D'Agostino[3], Zijiao Zhang[4], Wendy J. Mack, Constantinos Sioutas[4] , Caleb E. Finch[2,3], William J. Mack[1], Hongqiao Zhang[3]

[1]Keck School of Medicine, University of Southern California, [2]Dornsife College, University of Southern California, [3]Leonard Davis School of Gerontology, University of Southern California, [4] Viterbi School of Engineering, University of Southern California, Los Angeles, CA 90089

Background: Air pollution is strongly associated with increased risk of dementia in multiple populations. Experimental animal exposures show neurotoxicity of diesel exhaust particles (DEP), which are components of air pollution from traffic-heavy locations and electric power generation. Experimental models include direct exposure to direct diesel exhaust and DEP from individual sources. We characterized the neurotoxicity of DEP from a standard source for dose response and time course in mice.

Methods: DEP from National Institute of Science and Technology (NIST 2975, single engine) was suspended in pure water and re-aerosolized for exposure of young male C57BL/6 mice [1]. Controls received filtered air. Dose responses were evaluated for 0, 25, 50, and 100 μg/m3 for 5 hr; mice were examined after 18 hr. The time course of DEP exposure at 100 μg/m3 was extended up to 8 wks/200 hr, for assays of inflammation, oxidative stress, microglia activation, and soluble amyloid peptides (Aβ). This study adhered to ethical compliance for animal research.

Results: DEP at 100 μ/m3 dose for 5 hr, but not lower doses, induced microglial activation, inflammation, and oxidative damage in corpus callosum, and inflammation in lungs. Exposure to DEP for /8 wks caused additional white matter damage

Cerebral cortex had increased Aβ38 and 42 peptides and microglia activation, particularly in cortical layer 4.
Conclusion: DEP exposure at 100 µg/m3 caused robust neuroinflammatory and oxidative responses in white matter with as brief exposures of 5 hr. Prolonged DEP exposure caused further white matter damage and increased soluble Aβ in cerebral cortex, and microglia activation in multiple brain regions. The readily available DEP NIST 2975 gives a robust model of air pollution PM for replicable study of neurotoxicity and mechanisms.

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[1] Morgan TE et al. (2011) Glutamatergic neurons in rodent models respond to nanoscale particulate urban air pollutants in vivo and in vitro. Environ Health Perspect 119, 1003-1009.

Exposure and pulmonary toxicity assessments of dusts from machined plastic nanocomposites

T Stueckle[1] , E Lee[1] , H Shane[1] , J Coyle[1] , K Roach[1] , M Orandle[1] , J Jensen[1], R Derk[1] , J Kwon[2], S Friend[1] , A Afshari[1] , RK Gupta[3], C Dinu[3], L Rojanasakul[1]
[1] *NIOSH, Morgantown WV; [2]KOSHA, South Korea; [3]West Virginia University, Morgantown, WV*

Current advanced manufacturing approaches use engineered nanomaterials (ENMs) as filler material for plastic nanocomposites (PNCs). Organomodified nanoclays(ONC)are multifunctional materials that provide enhanced strength, barrier function, and other user defined properties. PNC manipulation and breakdown are expected across their lifecycle resulting in unknown inhalation hazards. These collective studies attempted to identify potential pulmonary health hazards across the ONC PNC lifecycle by identifying those PNC physicochemical properties that drive adverse pulmonary responses. Cloisite 93A and Cloisite 25A,two different ONCs, were embedded in polypropylene at different percent loadings(0%, 1%, and 4%)and then sanded to generate and characterize airborne particulate. ONC type and percent loading influenced PNC strength and toughness which aligned with particle release. 1% ONC PNCs produced greater total particle number and respirable mass concentration than 4% and 0%PNCs.Next, female Balb/C mice were exposed via six repeated aspirations to pristine Cloisite 93A (16.7 or 41.7 µg), 0%Cloisite 93A PNC machining dust, or1% Cloisite 93A PNC dust (50 or 150 µg). Animal procedures were reviewed and approved by the NIOSH IACUC. Bronchioalveolar lavage (BAL) and lung tissue samples were collected up to 28 days post-final exposure to evaluate inflammatory response.Mouse lungs exposed to high dose Cloisite 93A displayed evidence of allergic airway disease, including perivascular and peribronchiolar infiltrates, nodular accumulations of lymphocytes, and PAS-positive goblet cells in the terminal bronchiole epithelium. In BAL animals exposed to high dose Cloisite 93A displayed elevation in neutrophils, CD4+ T cells, and dendritic cells(CD11b+and CD86+/CD103+), which correlated with Th2-mediated inflammatory cytokine profile and pathological observations. 0%and 1% Cloisite 93A PNC dusts showed minimal multifocal granulomas,occasional alveolar histiocytosis, and minimal differences in inflammatory indices. In summary, repeated pulmonary exposure to ONCs early in their lifecycle poses a potential chronic inflammatory lung disease risk. This approach is currently expanding into other types of PNCs and use scenarios to identify common physicochemical characteristics that drive pulmonary toxicity.

POSTER ABSTRACTS

Exposure to Arsenic and Uranium in Drinking water alters Gastrointestinal Health

Aaron S. Romero[1], Fredine T. Lauer[2], Sebastian Medina[2], Scott W. Burchiel[2], and Eliseo F. Castillo[1]
[1] Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of New Mexico Health Sciences Center; [2] Department of Pharmaceutical Sciences, University of New Mexico Health Sciences Center

Metal exposure poses a serious environmental threat to the Native American community in New Mexico. From 1944 - 1986, the United States extracted 4 million tons of Uranium and other metal ores from native Navajo territories and consequently left over 10,000 abandoned mines. This mining and subsequent abandonment of mines caused random dispersion of heavy metals, windblown dust dispersal, and the contamination of drinking water with arsenic and uranium. This gastrointestinal exposure can have detrimental effects on gut health. We sought to understand how these heavy metals influenced the gut microenvironment. Metagenomic analysis of stool samples collected from mice exposed to both uranium and arsenic had alterations in α-diversity as well as changes in specific bacterial phyla and species that have differences in arsenic-related pathways. An oxidative metabolic state in mature intestinal epithelial cells (IEC) is critical for intestinal homeostasis and a diverse gut microbiota. This metabolic state is highly energy dependent and this energy is mainly derived from oxidative metabolism and fatty acid oxidation that subsequently preserves an obligate anaerobic microbial community. Thus, we furthered assessed if the heavy metals affected the metabolic state of IEC. Exposing the colonic cell line, Caco-2, to metals particles derived from environmentally relevant locations showed a higher glycolytic phenotype as indicated by higher basal glycolysis and higher compensatory glycolysis compared to controls. These data highlight the impact of metal exposure on intestinal barrier metabolism which influences the gut microbiota. Numerous metabolic disorders such as metabolic syndrome, type II diabetes, and non-alcoholic fatty liver disease have been linked to GI health and the gut microbiota. Taken together our data shows metal exposure causes alterations in the gut which may be a predisposing factor for metabolic disorders such as metabolic syndrome, type II diabetes, and non-alcoholic fatty liver disease.

Genomic basis for individual differences in susceptibility to the neurotoxic effects of diesel exhaust

Alexandra Noël[1], David G. Ashbrook[2], Fuyi Xu[2,6], Stephania A. Cormier[2], Lu Lu[2], James P. O'Callaghan[4], Shyam K. Menon[5], Arthur L. Penn[1], Byron C. Jones[2]
[1]Department of Comparative Biomedical Sciences, Louisiana State University, Baton Rouge, LA, USA; [2]Pennington Biomedical Research Center, Baton Rouge, LA, USA; [3]Department of Genetics, Genomics, and Informatics, University of Tennessee Health Science Center, Memphis, TN, USA; [4]Molecular Neurotoxicology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, Centers for Disease Control and Prevention, NIOSH, Morgantown, WV USA; [5]Department of Mechanical and Industrial Engineering, Louisiana State University,Baton Rouge, LA, USA; [6]School of Pharmacy, Binzhou Medical University, Yantai 264003, Shandong, China

Air pollution is a known environmental health hazard. A major source of air pollution includes diesel exhaust (DE). Initially, research on DE focused on respiratory morbidities; however, more recently, exposures to DE have been associated with neurological disorders and neurodegeneration. In this pilot study, we investigated the effects of sub-chronic inhalation exposure to DE on neuroinflammatory markers in two inbred mouse strains of both sexes, including whole transcriptome examination of the medial prefrontal cortex. We exposed aged male and female C57BL/6J (B6) and DBA/2J (D2) mice to DE, which was cooled and diluted with HEPA-filtered compressed air for 2 hours/day, 5 days/week, for 4 weeks. Control animals were exposed to HEPA-filtered air. The prefrontal cortex was harvested and analyzed for proinflammatory cytokine gene expression and transcriptome-wide response by RNA-sequencing. We observed differential cytokine gene expression between strains and sexes in the DE-exposed vs. control-exposed groups for Il1b, Tnfα, and Il6. We identified 150 differentially expressed genes between air and DE groupswhichrelated to natural killer cell-mediated cytotoxicity per Kyoto Encyclopedia of Genes and Genomes pathways. Overall, our data show differential strain-related effects of DE on neuroinflammation and neurotoxicity and demonstrate that B6are more susceptible than D2 to gene expression changes due to DE exposures. These results are important because B6 mice are often used as the default mouse model for DE studies and strain-related effects of DE neurotoxicity warrant expanded studies.All procedures were approved by the Louisiana State University Institutional Animal Care &Use Committe

Does inhalation of tungsten particulates affect the heart and increase the risk of cardiovascular disease?

Alicia M. Bolt[1], Sage Templeton[1], Charlotte M. McVeigh[1], Russell Hunter[1], Guy W. Herbert[1], Edward B. Barr[1], Barry E. Bleske[2], Matthew J. Campen[1]
[1]University of New Mexico, College of Pharmacy, Department of Pharmaceutical Sciences, [2]Department of Pharmacy Practice & Administrative Sciences, Albuquerque, NM, 87131

Inhalation of tungsten particulates is a relevant route of human exposure, especially in occupational settings where airborne tungsten concentrations have been reported as high as 3.1 mg/m3. In the hard metal manufacturing industry exposure to tungsten particulatesleads to increased incidence of interstitiallungdisease, characterized by pulmonary inflammation and fibrosis.In vivostudieshavealsoshown that inhalation of tungsten and tungsten-mixed metal alloy particulatesinduces inflammation and fibrotic changes in the lungs leading to impairedlung function. However,the systemic consequencesofthis pulmonary damagehave not been investigated.Multiple epidemiological studies have found an association between urinary tungsten levels and increased incidence and/or mortality of cardiovascular diseases. However, no studies have investigatedhow tungsten exposure affects the heart of the risk of developing cardiovascular disease.Using a whole-bodyinhalation exposure system 4, 4-hour exposuresto tungsten metal particulates (1.5± 0.32mg/m3; <1 μm),over the course of two weeks,resulted in changes in cardiac function including a decrease in cardiac output and an increase inthe amount of work required by the atria to fill the heart (A'). E'/A' ratio, an indicator of diastolic relaxation,also showed a decreasing trend.Gene expression profiling of tungsten-exposed hearts revealed a significant up-regulation in multiple pro-inflammatory, cardiac remodeling, and oxidative stress genes. These data strongly

suggest that subacute exposure to tungsten results in early signs of diastolic dysfunction including stiffening of the ventricle walls, diminishing filling volume, and potentially requiring increased atrial workload to fill the heart. Functional findings are in parallel with generation of cardiac ROS, inflammation, and early fibrotic changes. Current work is focused on determining how chronic exposure to tungsteneffectson cardiac function andthedevelopment of cardiovascular disease,and the role oftungsten-mediatedinflammation in disease pathogen

Inhalation of combustion-derived environmentally persistent free radicals causes vascualr enfothelial imjury mediated via AHR activation in alveolar type-2 pneumocytes

Ankit Aryal[a], Ashlyn C. Harmon[a], Lavrent Khachatryan[b], Pratiti Chowdhury[a], Alexandra Noël[a], Arthur Penn[a], Stephania Cormier[a], Huijing Xia[c], Kurt Varner[c] and Tammy R. Dugas[a]
[a]Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, [b]Department of Chemistry, Louisiana State University, [c]Department of Pharmacology and Experimental Therapeutics, School of Medicine, LSU Health Sciences Center

Particulate matter containing environmentally persistent free radicals (EPFRs) is formed by incomplete combustion of organic pollutants during thermal remediation. Initial studies used laboratory-generated EPFRlo: (1.5e16 radicals/g particles) and EPFRhi: (1.0e18 radicals/g) at 250 µg/m3 to investigate their effects on vascular endothelium and pulmonary oxidative stress in C57BL/6 male mice. These studies demonstrated that EPFRs inhalation results in an increase in endothelin-1 (ET-1) and intracellular adhesion molecule-1 (ICAM-1) at high but not low radical concentrations, and genes associated with AhR activation (Cyp1a1/1b1) were upregulated in the lungs. We also identified that AhR activation was significantly increased in AT-2 pneumocytes after EPFRhi exposure. We hypothesized that inhalation of EPFRs leads to vascular endothelial dysfunction via activation of AhR in AT-2 cells in a radical-dependent manner. To address our hypothesis, AhR was knocked down in AT-2 pneumocytes in male and female mice before exposure to filtered air (FA), EPFRlo, or EPFRhi for 4h/d for one and five days. Plasma ET-1 remained unchanged between KO and WT mice exposed to FA and EPFRlo; however, ET-1 was significantly decreased in AhR KO mice versus WT when exposed to EPFRhi for one day. Over the 5d exposure (4hr/d), we found an increase in mean arterial pressure in WT mice but not in AhR-deficient mice exposed to EPFRhi, supporting our hypothesis that AhR deficient mice are protected from endothelial injury due to EPFRhi exposure. Together, these data suggest that EPFR exposure promotes AhR activation in AT-2 pneumocytes, which results in vascular endothelial dysfunction, likely at the air-blood interface.

Sugarcane Ash and Sugarcane Ash-Derived Silica Nanoparticles Alter Mitochondrial Function and Metabolic Activity in Human Proximal Tubular Kidney Cells

Arthur D. Stem, Keegan L. Rogers, and Jared M. Brown
Department of Pharmaceutical Sciences, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of Colorado, Anschutz Medical Campus, Aurora, CO, 80045

Multiple epidemics of chronic kidney disease of an unknown etiology (CKDu), primarily in young healthy agricultural workers, have emerged in agricultural communities around the world. It is proposed that heat stress, dehydration and/or toxicant exposures may be a cause of this emerging disease. We have hypothesized that the harvest and burning of sugarcane leading to inhalation of sugarcane ash may contribute to development of CKDu. Sugarcane stalks consist of ~80% amorphous silica and we have demonstrated that following burning of sugarcane, nano-sized silica particles (~200 nm) are generated. To determine what effect such exposures have on kidney cells, a human proximal convoluted tubule (PCT) cell line (HK-2) was subjected to treatments ranging in concentration from 0.025 µg/mL to 25 µg/mL of sugarcane ash, desilicated sugarcane ash, sugarcane ash derived silica nanoparticles (SAD particles), or manufactured pristine 200nm silica nanoparticles. Following 6 to 48 hours of exposure, mitochondrial activity was analyzed via MTS assay and found to be significantly reduced when exposed to SAD particles at concentrations 2.5 µg/mL or higher. Mitochondrial membrane potential was investigated with a JC-1 assay, indicating depolarization at 6 hours following sugarcane ash exposure. Alterations to oxygen consumption rate (OCR) and pH changes were investigated with a Seahorse XF Analyzer and viability was determined with a plasma membrane integrity/esterase activity fluorescence assay. Significant changes to cellular metabolism occurred across treatments as early as 6 hours following exposure. While treatment with SAD particles greatly reduced normalized OCR, treatment with sugarcane ash and desilicated sugarcane ash increased OCR. Despite apparent shutdown of mitochondrial activity following exposure to SAD particles, treatment with FCCP (an uncoupling agent that maximizes mitochondrial oxygen consumption) yielded a massive increase in mitochondrial respiration in all treatment groups. Such changes indicate that exposure to sugarcane ash and its derivatives can promote mitochondrial dysfunction and alter metabolic activity of human PCT cells.

Combustion Derived Particulate Matter Exposure Exacerbates Influenza Infection by Inhibiting IL22 Production in Newborn Mice

Avinash Kumar, Stephania A. Cormier
Louisiana State University, Baton Rouge, LA; and, Pennington Biomedical Research Center, Baton Rouge, LA

Combustion-derived particulate matter (PM) containing environmentally persistent free radicals (EPFRs) are one of the major pollutants contributing to ambient air pollution. These are emitted during combustion and thermal processing of hazardous waste and organic materials. Inhalation of atmospheric PM is associated with increased respiratory diseases severity in infants. We previously reported that early-life exposure to PM containing EPFRs damages the lung epithelium and suppresses immune responses to influenza virus (Flu) infection, thereby enhancing Flu severity. Maintenance of lung epithelial layer during influenza virus infection is of critical importance to limit lung damage and pathogen dissemination. Interleukin 22 (IL22) is a member of IL10 family of cytokines and predominantly produced by innate and adaptive T cells, which help in resolving lung injury following Flu infection. In the current study, we determined the effects of EPFR exposure on pulmonary IL22 responses using our neonatal mouse model of Flu infection. Exposure to PM containing EPFRs resulted in an immediate (0.5-1-day post-exposure) increase in IL22 expression in the lungs of C57BL/6 neonatal mice; however, this IL22 expression was not maintained and failed to increase with either continued exposure to PM or subsequent Flu infection of PM-exposed mice. This contrasts with increased IL22 expression in age-matched mice exposed to vehicle and Flu infected. Activation of the aryl hydrocarbon receptor (AhR), which mediates the induction and release of IL22 from immune cells, was also transiently increased with PM exposure. The microbiome plays a

major role in maintaining epithelial integrity and immune responses by producing various metabolites that act as ligands for AhR. Exposure to PM induced lung microbiota dysbiosis and altered the levels of indole, a microbial metabolite. Treatment with recombinant IL22 or indole-3-carboxaldehyde (I3A) prevented PM associated lung injury. In addition, I3A treatment also protected against increased mortality in Flu-infected mice exposed to PMs. Taken together, these data suggest that exposure to PM containing EPFRs results in failure to maintain IL22 levels and an inability to induce IL22 upon Flu infection. Insufficient levels of IL22 may be responsible for aberrant epithelial repair and immune responses, leading to increased Flu severity in areas of high PM.

Species Differences in TRPM8 Affect Pulmonary Sensitivity to Chemical and Particulate Matter Agonists

Cassandra E. Deering-Rice, John G. Lamb, Christopher A. Reilly
University of Utah

Exposure to air pollution is often unavoidable and mechanisms leading to short- and long-term adverse effects are not fully understood. Particulate matter (PM) in air pollution has many origins. Coal fly ash (CFA), diesel exhaust, cigarette smoke, calcium oxide, and wood smoke are examples of PM. Activation of transient receptor potential (TRP) channels is one mechanism by which PM can trigger adverse effects in the lungs, including asthma exacerbation. Notably, TRPA1, TRPV1 and TRPM8 have been linked to the initiation of inflammation and cell injury. Initial findings using human airway epithelial cells have shown that activation of TRPM8 by CFA and CaO nanoparticles triggers cytokine gene expression and secretion, which was not replicated in mice. We hypothesized that species-specific differences in TRPM8 activation by PM may underlie this difference. Alignment of human and mouse TRPM8 revealed three differences in the amino acid sequence of the putative pore-loop domain. Mouse and human TRPM8 plasmids, and mutants thereof, were prepared and transiently transfected into GcAMP6-overexpressing HEK-293 cells; calcium flux assays were then performed. Mutation of mouse Trpm8 to incorporate the corresponding human residues conferred varying levels of sensitivity to PM agonists, with the S921 G and S927 A mutants resulting in comparable responsiveness to the human receptor using CFA and CaO as agonists. Reciprocal mutations confirmed amino acid 927 as critical PM for sensitivity. Transgenic "humanized" C57BL/6 mice were engineered to express Trpm8 S927A, and the effects on pulmonary inflammation, mechanics and lung morphology were evaluated basally and following oropharyngeal aspiration delivery of CFA. In general, humanized mice demonstrated higher basal levels of pro-inflammatory IL6 and Cxcl8, altered respiration and lung mechanical properties consistent with an inflamed lung, and evidence of alveolar edema relative to wild-type C57Bl/6 mice, which were comparably affected by CFA exposure. Follow-up studies using isolated mouse DRG neurons and primary mouse tracheal epithelial cells complimented the in vivo data and suggested a role for CFA-sensitive TRPV1 in regulating responses to CFA in vivo. Identification of specific PM-sensing sites on TRP channels furthers our knowledge of mechanisms by which TRPs are activated by PM, reveal potential limitations in standard animal models for assessing roles of TRP channels in particle toxicity and translation of findings to humans, as well as advance our understanding of mechanisms by which PM promote lung inflammation, injury, and disease. Support: ES017431, ES027015.

Graphic Warning Labels Potentially Reduce Genotoxicity in Buccal Epithelial Cells Associated with Waterpipe Smoking

Chieh-Ming Wu[1], Amy Ferketich[2], MarielleBrinkman[2], Michael Pennell[3], and Olorunfemi Adetona[1]
[1]Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH. [2]Division of Epidemiology, College of Public Health, The Ohio State University, Columbus, OH. [3]Division of Biostatistics, College of Public Health, The Ohio State University, Columbus, OH.

Background: FDA-mandated health warning labels, sometimes along with graphic images, have been linked to reduced tobacco use or even cessation. Applying warning labels could help lower the amount of smoke inhaled by smokers and thereby reduce local/systemic effects. To date, the evaluation of the effectiveness of warning labels on waterpipe smokers' health is not well documented. This study aims to determine if the use of warning labels could reduce the acute and longer-term genotoxic effect in exfoliated buccal cells using the single cell gel electrophoresis (i.e., comet assay).
Methods: Twenty-four participants were recruited and randomly assigned to two groups: Blank Label (Group 1, N=12) and Graphic Warning Label (Group 2, N=12). Each group had two smoking sessions and one 3-month follow-up visit. While the blank label was used throughout the visits in Group 1 and the first visit in Group 2, the graphic warning label was used during the second visit for Group 2 only. Buccal epithelial cells were collected before and after the smoking session as well as during the follow-up visit. The cells were washed, fixed on frosted slides, dipped in lysing solution overnight and then subjected to electrophoresis under alkaline conditions (pH>13). Comet parameters (i.e., tail length, % tail DNA, tail moment, and olive tail moment) were scored using the ImageJ OpenComet. Changes in the parameters between pre-and post-smoking and the first and second visit were tested using one-sample and paired t-tests, respectively.
Results and Conclusions: Overall, mean olive tail moment increased significantly after waterpipe smoking (p-value=0.02). Genotoxicity measures increased less at the second visit (warning label) compared to the first visit (blank label) in Group 2 and compared to those measured at the second visit (blank label) for Group 1, but these differences were not statistically significant. Following 3 months after the visit, the status of genetic damage in both groups returned to similar levels at the beginning of the study. The results indicate that waterpipe smoking increases oral genotoxicity and that health warning label might mitigate such effect.

Inhalation toxicity of copper sulphate pentahydrate and dicopper oxide

Craig Poland[1,2], Sue Hubbard[3], Len Levy[4], Yamini Gopalapillai[5], and Carol Mackie[1]
[1]Regulatory Compliance Limited, Loanhead, Edinburgh, UK [2]Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK [3]SAHCo Ltd, Chester CH3 7JW, UK [4]Cranfield University, Cranfield, U [5]Material Stewardship, International Copper Association, Washington, DC, USA

Inhalation exposure to copper may occur in a variety of occupational environments ranging from the mining and refining of copper metal to the production and use of copper compounds. To investigate the potential inhalation effects of copper exposure, a 14-day range finding study was conducted using two copper compounds, differing in solubility and representative of copper substances in large-scale production/use. Crl:CD(SD) rats were repeatedly exposed to aerosols of dicopper oxide (Cu2O) or copper sulphate pentahydrate (CuSO4.5 H2O) at normalised

copper doses of 0.18, 0.71, 1.78 and 8.9 mg/m3 Cu. There was a dose-related increase in lung macrophages with an acute influx of polymorphonuclear leukocytes (neutrophils) at ^{3}1.78 mg/m3 Cu for both compounds whilst only CuSO4.5 H2O exposure resulted in epithelial cell hyperplasia. This epithelial response may reflect the rapid dissolution of CuSO4.5 H2O in lung lining fluid leading to a release of copper ions at the epithelial surface whilst Cu2O is relatively indissolvable at neutral pH.
The inhalation effects were studied in more detail during a 28-day study with Cu2O at doses of 0.2, 0.4, 0.8 and 2.0 mg/m3 Cu2O following OECD TG 412. To assess the temporal response with ongoing exposure, satellite groups were exposed for 1-, 2- or 3- weeks in addition to a 13-week post-exposure recovery period group. The response was characterised by localised alveolar histiocytosis and neutrophil influx with no evidence of epithelial hyperplasia or fibrosis in the lung and no systemic effects. The satellite groups showed that this acute inflammatory response peaked between weeks 1-3 and reduced thereafter despite ongoing exposure. This biphasic response may indicate adaptation and a shift towards a pro-resolution response with all biomarkers returning to control levels during the post-exposure recovery period. Overall, these results show that repeated exposure to copper compounds results in a controlled acute cellular response with no associated pathology and which fully resolved after the cessation of exposure.

Acrolein, a major constituent of combustion emissions, induces sexually dimorphic neuroendocrine and metabolic dysfunction

Devin I. Alewel[1], Thomas W. Jackson[1], Andres R. Henriquez[1], Mette C. Schladweiler[2], Samuel A. Vance[1], Stephen H. Gavett[2], Anna A. Fisher[2], Rachel D. Grindstaff[2], Paul A. Evansky[2], Urmila P. Kodavanti[2]
[1]ORISE, EPA, Durham, NC, USA; [2]PHITD, CPHEA, EPA, Durham, NC, USA

Anthropogenic and wildfire smoke emissions are the most prominent contributors to present-day air pollution. Acrolein, a major constituent of these emissions, is on EPA's priority list of hazardous air pollutants. It is known to cause airway irritancy and inflammation; however, neuroendocrine and systemic health effects are not well characterized. Moreover, few studies have addressed potential sexually dimorphic responses to inhaled stressors. Here, 12-week-old male and female Wistar-Kyoto rats underwent acrolein nose-only inhalation in incremental concentrations (0, 0.1, 0.316, 1ppm) for 30 min (for assessment of ventilatory changes), followed by a 3.5hr exposure at 3.16ppm (n=8/group). We assessed nasal and pulmonary lavages (NALF and BALF), circulating pituitary, adrenal, thyroid and gonadal hormones, and other hallmarks of peripheral stress. We further analyzed circulating biomolecules through serum metabolomics. Acrolein induced potent nasal but not pulmonary injury and vascular leakage (increased NALF total protein, albumin, and LDH activity) in both males and females. However, the inflammatory response was apparent in both nasal and pulmonary airways but only in males. Acrolein caused robust corticosterone release only in males, also displaying glucocorticoid-like effects of lymphopenia and hyperglycemia. Further, serum pituitary hormone levels of triiodothyronine, prolactin, and testosterone were greatly diminished in acrolein-exposed males. Metabolomic analysis of both sexes revealed increased lipolysis, muscle protein catabolism, and shifts in mitochondrial respiration markers associated with changes in steroid metabolism in males but not in females. In conclusion, acrolein exposure induces neuroendocrine stress reactions that are linked to changes in circulating metabolites reflective of alterations in metabolic processes within liver, adipose, and muscle tissue. Moreover, these changes are sex-specific, where males show increased susceptibility to systemic stress. This prompts further investigations into neuroendocrine and sex-specific mechanisms that govern peripheral stress from exposure to reactive air pollutants (Does not reflect US EPA policy).

Animal Care and Use Declaration:
US EPA Institutional Animal Care and Use Committee approved protocol prior to experiments.

Surface engineering strategy to reach a safer and more performing profile of TiO2-NPs as sunscreen UV filters

Elena Cesa[1,2,7], Paola Ziosi[2], Anna Luisa Costa[3], Dario Fornara[4], Mustafa Culha[5], Giuseppe Valacchi[1,7], SilviaVertuani[1,2], Stefano Manfredini[1,2]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; [2]Ambrosialab srl, Ferrara, Italy; [3]ISTEC-CNR, Faenza, Italy [4]Lamberti SpA, Albizzate, Italy [5]Sabanci University Nanotechnology Research and Application Center, Istanbul, Turkey [7]PHHI, Animal Science Dept., NC State University, NC, USA

TiO2 nanoparticles(TiO2-NPs) are used in many applications such as in biomedicine and cosmetics due to its chemical inertness properties, high refractive index, low cost and advantageous surface properties. With their wide spread use, TiO2-NPs toxicity has assumed increasing relevance and concerns about both environment and human exposure through ingestion, dermal penetration, or inhalation route. Their biological effects and the cellular response mechanisms are still not completely elucidated and thus a deep understanding of the toxicological profile is required. In this study, we have focused on the most significant route of exposure: skin contact, with sunscreen containing TiO2-NPs as UV filters. Because the main mechanism underlying the toxicity potentially triggered by TiO2-NPs seems to involve the reactive oxygen species (ROS) production, the extent and type of cell damage strongly depend on chemical and physical characteristics of NPs, including size, surface composition and photo-activation. Following a "Safe and Sustainability by Design" (SSbD) approach to mitigate their photo-toxicity by preserving their UV filtering capacity, we have designed a surface modification on TiO2-NPs with a suitable and eco-friendly molecule obtained by a biotechnology approach involving fermentation of by-products from the food industry. After the optimization procedures, the characterization of obtained species was conducted by using spectroscopic, thermal and images techniques, as well as the evaluation of photocatalytic effects and cytotoxicity. A dependence of in vitro and ex vivo responses by the molecular structures used to coat their surface was found. The new coated UV filters have shown different properties and benefits: a higher SPF (ISO24443:2012), a better cell compatibility (LDHassay) and a strong reduction of photocatalytic activity (Acidblue9test). In conclusion, the implementation of SSbD approach on TiO2-NPs surface results to be fruitful in optimizing UV protection capacity, obtaining more sustainable and safer UV filters.

Effect of UV-Exposure on the Uptake and Toxicity of Respirable Polyester Fiber Particles

A. Neidhart[1], T. Howard[2], M. Spilde[2],A. Maestas-Olguin[2],A.Benavidez[2], A. Brearley[2], S.Shoultz[2], A. Abudushalamu[2], G. Herbert[1], J. Gonzalez-Estrella[3], J. Cerrato[2], M. Campen[1], and E. El Hayek[1]

[1]University of New Mexico College of Pharmacy, Albuquerque, New Mexico; [2]University of New Mexico, Albuquerque, New Mexico; and [3]Oklahoma State University, Stillwater, Oklahoma

Air pollution and climate change are listed among the important risk factors that may drive the development of respiratory diseases by increasing oxidative stress and inflammation in the lung. Climate change can increase climatic stress factors (i.e. UV radiation and temperature extremes) and cause oxidative alterations to the surface physicochemistry of air pollutants. Respirable microplastics and fibers in air particulates may be subjected to such environmental weathering. The documentation of occupational respiratory diseases in synthetic textile workers and the recent evidence on the presence of fibers in human lung tissue exacerbate the concern on fibers cytotoxicity and inflammation in the lung. Here, we evaluated the physicochemical characteristics and toxicity of fiber particles from a fleece polyester fabric before and after UV weathering by integrating microscopy, spectroscopy and cytotoxicity. Raman spectroscopy confirmed that the chemical structure of leached particles from the fleece blanket matches with polyester microfibers. Scanning electron microscopy energy-dispersive spectroscopy and Raman spectroscopy revealed the increase of the concentration of metals impurities (i.e. titanium and silica) on the surface of UV-aged particles and the decrease in the intensity of allenes group respectively. Carbonyl, ketone, and carboxylic functional groups increased on the near-surface region of UV-aged particles as indicated by X-ray photoelectron spectroscopy. Both fresh and UV-aged fibers of respiratory sizes induced dose-dependent cytotoxicity. UV radiation amplified fiber particles cytotoxicity by increasing 10 % the cell mortality at 500 µg/ml particles concentration, in comparison to fresh fibers. Transmission electron microscopy identified the intracellular translocation of UV-aged particles at 50 µg/ml particles concentration. Our study highlights the importance of understanding the environmental health risks from fiber particles exposure and their implications in the inflammatory mechanisms in the lung.

Direct Evidence of Metabolic Interactions between PBDEs and Gut Microbes: an In Vitro Metabolomics Study

Yan Jin [1], Kyle Kim [2] , Julie De Laloubie [1,3] , Kris Weigel [2] , Julia Yue Cui [2] , Haiwei Gui [1]

[1]Center for Translational Science, Florida International University, Port St. Lucie, FL

[2] Department of Environmental and Occupational Health Sciences, University of Washington, WA

[3] School of Engineering Sciences, Polytech Clermont-Ferrand, Clermont Auvergne University, France

Polybrominated diphenyl ethers (PBDEs) were extensively used as flame retardants in various factory products and are still persistent in the environment after they were banned to use. Recently, we showed in various in vivo studies that gut microbiome dysbiosis is involved in PBDEs-induced toxicity; however, it is unknown whether PBDE exposure directly impacts the metabolism of the gut microbiome. In this in vitro study, we used mass spectrometry-based metabolomics approaches to investigate the metabolic interactions between 2 common PBDE congeners (2,2′,4,4′-tetrabromodiphenyl ether [BDE-47] , 2,2',4,4',5- p entabromodiphenyl ether [BDE-99]) and selected gut microbe species (Akkermansia muciniphila [AKK] and Clostridium scindens [CS]) that are known to be involved in metabolic diseases, as well as Escherichia coli. (EC), an established control microbe. All microbes were individually cultured in an anaerobic workstation. These bacterial strains were exposed with BDE-47 and BDE-99 of different concentrations (0, 10 µM , or 100 µM). Pathway-specific targeted LC-MS/MS metabolomics was used to examine ~300 aqueous metabolites from ~30 metabolic pathways of biological significance. Our results suggest that both BDE-47 and BDE-99 significantly altered metabolic profiles in all these 3 bacterial strains. For example, in the principal component analysis (PCA) score plot (Figure 1), CS after BDE-47 exposure is clearly separated from controls in a dose-dependent manner. Additionally, metabolomics results revealed that BDE-47 and BDE-99 caused different metabolic responses in these gut microbes. For example, CS had increased lactate and decreased kynurenine after BDE-99 exposure, while BDE-47 induced decreased 2-pyrrolidinone and increased urocanic acid in CS . Furthermore, various altered metabolites were found significant in multiple metabolic pathways, especially in glycolysis, TCA cycle, and tryptophan metabolism. A total of 60 metabolic features were determined to distinguish potentially disturbed metabolite markers of BDE-47 and BDE-99 exposure. In conclusion, our findings provide possible biomarkers of toxic effects induced by BDE-47 and BDE-99 and elicit a deeper understanding of the metabolic mechanisms that could be validated in further in vivo studies.

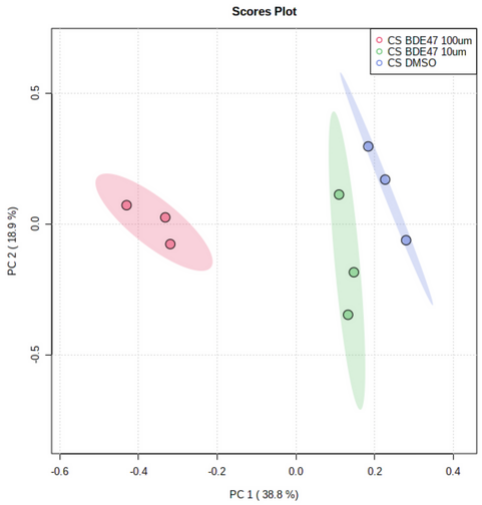

Figure 1. The PCA score plot of CS samples after BDE-47 exposure

Role of Lipid Metabolism in Particulate Matter-Induced Lung Inflammation and Injury

Hannah Lovins, Michael Yaeger, Katelyn Dunigan Russell, Grace Hutton, Evangeline Schott, Drew Miller, Matthew W. Gorr, Loren E. Wold, Kymberly M. Gowdy

Ohio State University, Columbus, OH

Particulate matter (PM) is a criteria air pollutant shown to increase morbidity and mortality fromchronic lung diseases. Fine PM (PM2.5) induces lung injury and inflammation, in part through activation of alveolar macrophages, as well as through the production of pro-inflammatory lipid mediators such as prostaglandins and leukotrienes. Recent studies have identified a novel class of lipid mediators, termed specialized pro-resolving mediators (SPMs) that resolve inflammation following injury. However, it is currently unknown if PM2.5 alters SPM production. From this, we hypothesize that PM2.5 induced lung

inflammation/injury is due to an imbalance in pro-inflammatory lipid mediators and SPM production.

To test our hypothesis, C57BL/6J male mice were exposed to either filtered air (FA) or concentrated PM2.5(mean daily PM2.5 exposure =78.0 ±11.1 µg/m3) 6 hours/day for 3 or 6 weeks. 24 hours post final exposure, mice were euthanized and bronchoalveolar lavage (BAL) fluid and lung tissue were collected to assess lung inflammation/injury and lipid metabolism via lipidomics. Air space macrophages were isolated from BAL for qPCR to determine the macrophage specific lipid metabolism response.

PM2.5 exposure induced lung injury/inflammation as demonstrated by an increased BAL neutrophilia and total BAL protein following 3 weeks of exposure. However, this increase in lung injury/inflammation was not noted after 6 weeks of PM2.5 exposure. Lung tissue lipidomics indicated no differences in SPM production between PM2.5 and FA at 3-or 6-weeks post exposure, whereas pro-inflammatory lipid mediators 9,10-DiHOME and 12,13-DiHOME were increased following 3 weeks of PM2.5 exposure. Additionally, airspace macrophages had a significant increase in expression of the lipid metabolizing enzyme, ALOX5 following 3 weeks of PM2.5 exposure and was not seen post 6 weeks of exposure.

Taken together, subchronic PM2.5 exposure induces lung injury/inflammation and the pulmonary production of 9,10-and 12,13-DiHOME production. Additionally, preliminary data indicate a macrophage specific change in lipid metabolism following PM2.5 exposure, which will be pursued in future studies. All experiments were performed in accordance with the Animal Welfare Act and the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals

Characterization of different aerosols using a novel thermal spray coating and inhalation exposure system

A Afshari, W McKinney, JL Cumpston, HD Leonard, JB Cumpston, M Jackson, S Friend, V Kodali, T Meighan, A Erdely, JM Antonini.
National Institute for Occupational Safety and Health, Morgantown, WV.

Thermal spray coating (TSC) involves spraying a metal coating product that is melted by extremely high temperatures and then applied under air pressure onto a surface. Large amounts of a complex metal aerosol comprised of Fe, Cr, Ni, and Zn are formed during the process, presenting a potentially serious risk to the operator. Information about the health effects associated with exposure to aerosols generated during TSC is lacking. Even less is known about the chemical and physical properties of these aerosols. The goal was to construct and characterize an automated TSC aerosol generator and animal inhalation exposure system that would simulate workplace exposures. An electric arc wire-TSC aerosol generator and exposure system was designed and separated into two areas: (A) an enclosed room with a closed spray booth where the spray coating occurred; (B) an exposure chamber with different particle characterization devices. The physicochemical properties of aerosols generated during electric arc wire-TSC using five different consumable wires were examined. The metal composition of each was determined by ICP-AES, including two stainless-steel wires [PMET720 (82% Fe, 13% Cr); PMET731(66% Fe, 26% Cr)], two Ni-based wires [PMET876 (55% Ni, 17% Cr); PMET885 (97% Ni)], and one Zn-based wire [PMET540 (99% Zn)]. The generated particles, regardless of composition, were poorly soluble, complex metal oxides and mostly arranged as chain-like agglomerates of primary particles in the ultrafine range (<100 nm). The MMAD as determined by a MOUDI was similar when comparing the generated particles from the different wires and ranged from 290-378 nm. To allow for continuous, sequential coating within the spray booth during a 2-hr testing period, a motor rotated the metal feedstock pipe to be coated in a circular and up-and-down direction. The spray gun was controlled by computer software and fired at programmed 1-sec intervals for up to a total of 12 sprays during the testing period. A targeted exposure chamber concentration of 25 mg/m3 was achieved and maintained during the 4-hr period, along with minimal fluctuations in relative humidity, temperature, and CO2 levels. This exposure system will generate continuous, controlled metal spray coating aerosols for future animal exposure studies.

In vivo Lung Toxicity associated with Boron Nitride Nanotubes with Different Purities

J.R. Roberts[1], X. Xin[1], K.A. Roach[1], M.B. Jakubinek[2], K.S. Kim[2], L. Bowers[1], M. Wolfarth[1], S.S. Leonard[1], D.W. Porter[1], S. Friend[1], A. Erdely[1], V. Kodali[1]
[1]NIOSH, Morgantown WV; [2]National Research Council Canada, Ottawa ON

Boron nitride nanotubes (BNNTs) are a high aspect ratio nanomaterial composed of hexagonal B-N in a multi-walled tube formation. Their material properties make them advantageous in applications in composites, energy storage, radiation and thermal shielding, as well as biomedical applications. The goal of this study was to assess the lung toxicity BNNTs with various purities in vivo. The National Research Council, Canada, provided three BNNT samples: a low purity as-produced sample with ~50% tubes (AP-BNNT), a purified sample with elemental boron removed (BR-BNNT), and a highly purified washed/filtered BR-BNNT sample (W2-BNNT). Hexagonal boron nitride (h-BN, <100 nm in diameter) was used as a control material for synthesis by-products. Samples were characterized for material properties including purity and size in powder form and in dispersion medium (DM, vehicle control). Purity (tube concentration) varied with W2> BR >AP with tube dimensions of geometric mean length of 1.68 µm ± geometric standard deviation (GSD) of 1.9 and a geometric mean diameter ± GSD of 0.017 µm ± 1.329 in powder form. Agglomerate sizes in DM ranged from ~285-350 nm for BNNT and h-BN. Male C57BL/6 mice were exposed by oropharyngeal aspiration to 4 or 40 µg of sample/mouse in DM or DM alone. Mice were euthanized at 4 h, 1 d, 7 d, 1 mo, and 3 mo post-exposure, lung lavage was performed to evaluate lung injury and inflammation on one group of animals. Lungs from a second group were collected for histopathology. Lavage parameters of lung injury and inflammation were significantly increased by the high dose of BNNTs at the early time points with W2>BR>AP and persisted in the W2 group up to 1 m post-exposure. h-BN produced low to no indicators of toxicity in lavage parameters measured. In lung tissue, inflammation and microgranuloma formation were noted with h-BN and BNNT exposures; however, severity was minimal to mild for all groups. Incidence and severity were greatest in the W2 group and began to resolve in all groups by 3 mo post-exposure. Recovery was fastest in the h-BN group. The results indicated that the tested BNNT samples induced acute toxicity and inflammation only at high concentration and the effects were more pronounced with increasing purity. The reference material used to represent one of the by-products of synthesis, h-BN, showed little toxicity relative to BNNT, suggesting effects where present may be due to BNNTs.

Ethical use of Animals: All animal studies were reviewed and approved by the CDC, Morgantown, WV IACUC in a PHS-assured, AAALAC-accredited facility in compliance with OLAW and The Guide for Care and use of laboratory Animals.
Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention.
Key Words:
Boron Nitride Nanotubes, Lung Toxicity, In Vivo
Animal Care and Use Declaration: US EPA Institutional Animal Care and Use Committee approved protocol prior to experiments.

The Influence of Variable Electronic Cigarette Coil Power on Lung Cell Health

Adragna J, Katsigeorgis M, Karey E, and Gordon T,
NYU Langone Health Center, New York, NY

The popularity of electronic cigarettes has grown over time, whereas their health effects remain poorly explored. Traditional animal models are costly, ethically problematic, and do not suffice for the exploration of human toxic exposures. The Calu-3 human derived adenocarcinoma cell line is a good compromise in vitro model. When cultured at air liquid interface (ALI), they form a polarized monolayer with tight junctions and a functional epithelial barrier. Using a Calu-3 ALI model, we investigated the effects of vaping coil power on the respiratory barrier. Calu-3 inserts were exposed to e-cigarette aerosol for 30 minutes between 8.5W and 20W. DNA damage increased for all groups in a power dependent manner. Respiratory barrier resistance and LDH release occurred at the highest power level. IL-8, a neutrophil recruitment factor, was upregulated for most groups. IL-6 was downregulated for all groups. There are other mixed significant effects among cytokines tested.

MacLEAP: Machine - Learning Approach for Recognition and Quantification of Carbon Content in Airway Macrophages

Chelin Hu[1], Shuguang Leng[2], and Xiaozhong "John" Yu[1]
[1]College of Nursing , [2]School of Medicine, University of New Mexico, Albuquerque, NM

Combustion-emitted particulate matter has been regarded as the most impactful and causal component of ambient PM2.5 for cardiopulmonary morbidity and mortality. Macrophage carbon load (MaCL) has been considered as a bio-effective lung dose of carbon-containing respirable particles, which is the major component of particulate matter in the ambient air. Manual scoring of MaCl is labor-consuming and scorer-dependent, with a limitation in large-scale epidemiological studies. The goal of this study is to develop a machine learning approach for Engulfed Carbon Particles (MacLEAP) that automatically recognizes and segments the macrophages from other cell types on the sputum images and quantifies the engulfed black carbon particles. MacLEAP is developed based on the free, open-source deep convolutional neural network Mask_RCNN. The state-of-the-art deep learning approach Mask_RCNN was adopted to train and recognize the macrophage and quantify nano-scale black carbon particles. A total of 357 bright-field Papanicolaou staining sputum images from 17 individuals of the Lovelace Smokers cohort were used in the training and validation of the models. We adopted a cascaded approach to develop both macrophage (Model 1) and carbon particle models (Model 2). We found MacLEAP yielded excellent intraclass correlation coefficient (ICC) scores with manual count results (0.98 for macrophage and 0.92 for carbon), exhibited greater robustness on challenging images, and required less repetitive trials in the model training steps. In conclusion, this study established a MacLEAP machine learning model, which provides a high-throughput and score-independent approach to quantify MaCL levels from thousands of slide images.

Magnetic Resonance Imaging Contrast Agents Induce Acute Tubular Damage a nd Gadolinium-rich Nanoparticle Formation in Renal Cortex

Joshua DeAguero[1,5], Tamara Howard[2], Adrian Brearley[3], John Watt[4], G. Patricia Escobar[1,5], Karol Dokladny[1,5], Brent Wagner[1,5]
[1]Kidney Institute of New Mexico, University of New Mexico Health Science Center, [2]University of New Mexico Health Science Center, [3]University of New Mexico Department of Earth & Planetary Science, [4]Center for Integrated Nanotechnologies, Los Alamos National Laboratory, [5]New Mexico Veterans Administration Health Care System

Gadolinium-based contrast agents (GBCAs) are a dynamic tool used to enhance magnetic resonance imaging (MRI) examinations. Because gadolinium itself is toxic, the metal is chelated with organic ligands for detoxification that enhance elimination. GBCAs cause systemic fibrosis. We have reported that GBCA exposure leads to the in vivo formation of intracellular nanoparticles in rodent kidneys. Similarly, our group has identified similar nanoparticles in human kidneys, with prior exposures to GBCAs. However, the chemical composition of these nanoparticles is unknown.
Male and female C57/BL6 mice were randomized to untreated (n = 20) or GBCA-treatment (n = 20) (Omniscan, 2.5mmol/kg, intraperitoneally, 20 doses). Kidneys were fixed, sectioned at 200nm, and placed onto carbon holey support grids. Human kidney samples were obtained from the University of New Mexico's Human Tissue Repository and were processed similarly for electron microscopy. Scanning electron microscopy and elemental analysis by energy-dispersive x-ray spectroscopy (XEDS) were performed. The animal experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee of the University of New Mexico.
Electron-dense nanoparticles and lipid droplets rimmed with electron-dense material littered renal epithelia in the GBCA-treated animals. XEDS line scanning revealed that the electron densities contained gadolinium, phosphorous, and calcium. Human kidney samples demonstrated nanoparticles akin to the treated animals. Elemental analysis showed that the nanoparticles were positive for gadolinium and phosphorous.
Systemic magnetic resonance imaging contrast agent treatment leads to the self-assembly of gadolinium-rich nanostructures in kidney tubular cells. These in vivo findings suggest that transmetallation or metabolism of the agent is occurring. Intracellular mineralization of gadolinium may be an initial mechanism for resultant disease. Speciating these precipitates may aid in prophylactic strategies and therapies for gadolinium-induced diseases.

Augmented kidney and liver gadolinium retention in experimental obesity

Brent Wagner[1], Abdul Mehdi Ali[2], Joshua DeAguero[1], G. Patricia Escobar[1], Karol Dokladny[1]
[1]Kidney Institute of New Mexico/University of New Mexico Health Sciences Center, [2]University of New Mexico Earth and Planetary Sciences Department

Background: Gadolinium is a heavy/rare earth (lanthanoid) metal extensively used in modern diagnostic medicine as an enhancer of magnetic resonance imaging (MRI) procedures. Exposure to gadolinium-based contrast agents can cause 'nephrogenic' systemic fibrosis (NSF), a medical condition

characterized by skin fibrosis associated with severe pain, burning, and itching leading to inhibition or loss of joint flexibility and movement. Our team has previously shown that gadolinium is detectable in both symptomatic and asymptomatic patients in the urine, hair, and nails after MRI contrast exposure. We have also pioneered mechanistic studies showing that C-C chemokine receptor 2 activated myeloid cells and fibroblasts mediate NSF. The kidney is the primary reservoir for gadolinium-based contrast even days after a single dose. However, gadolinium retention in obese animals has not been examined.

Methods: Mice (males and females) were randomized to a 60 kcal% fat diet (n=14) ad libitum (20 kcal% protein and 20 kcal% carbohydrates; Research Diets, Inc; D12492i) or control chow (19% protein, carbohydrates 47%, and fat 6.5 %; Tekland Diets; 2020x; n=8) for 18-20 weeks. Then, the groups were sub-divided into untreated (n=8) and gadolinium-based contrast agent-treated (Omniscan or Dotarem 2.5 mM) (n=14) subgroups 5 days a week for 4 weeks. Tissues were excised and snap-frozen in liquid nitrogen. On average, 15 mg of tissue were digested and gadolinium concentrations were quantified using PerkinElmer NexION 300D Inductively Coupled Plasma Mass Spectrometry (ICP/MS) with a detection limit of 0.01 ppb.

Results: Both males and females on HFD show an increased gadolinium accumulation in tested organs regardless of the MRI contrast agent. Regardless of sex, Omniscan or Dotarem increased tungsten retention in the organs. Renal calcium retention was increased by HFD. Dotarem treatment suppressed HFD-induced renal calcium retention (both sexes). Zinc accumulation was increased in males on HFD treated with Dotarem.

Conclusions: Our data indicate that obesity promotes gadolinium retention in the kidney and liver. Future studies are needed to delineate the cellular mechanisms leading to the augmented gadolinium accumulation in obese animals and to demonstrate associated pathological consequences.

Ex vivo to in vivo extrapolation for respiratory toxicity of inhalable substances

Schwarz K, Winterberg D, Schaudien D, Obernolte H, Sewald K, Braun A

Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

Prediction of in vivo toxicityfromin vitro/ex vivostudies could substantially contribute to a faster and more effective drug development as well as toanimal welfare by reducing the numbers of animal studies. This howeverremains challenging due to the complex biological processesand interactions, differences in the exposure protocols (concentration, exposure time, repeated exposure)as well as the dosimetry. The model of the Isolated Perfused Rat Lung (IPL) providing a fully intact organ system with cellular, structural and functional integrity including pulmonary air:blood:barrier function and circulation is an suitable tool due to this proximity to the in-vivo situation. The aim of the present works was to apply Isolated Perfused Rat Lungs to estimate respiratory toxicity of Nafamostat mesylate prior to a regulatory subacute toxicity study.Ventilated and perfused lungs from rats were exposed to respirable Nafamostat mesylate aerosols in a dose escalation scheme.Using the MPPD (Multiple Path Particle Dosimetry) model, the regional deposited doses were determined. Measurement of tidal volume allowed for the on-line assessment of the viability of the lungs. In addition, Precision-Cut-Lung-Slices of the IPLs were prepared and analyzed for viability by confocal microscopy after LIVE/DEAD stain. The resulting effect level data obtained ex vivo were compared to the corresponding data from a 28-day subacute in vivo inhalation studyin rats.Exposure to Nafamostat mesylate aerosols resulted in a dose-dependent decrease in lung functionfor high concentrations. A No Observed Adverse Effect Level (NOAEL) and a Lowest Observed Adverse Effect Level (LOAEL) could be derived.In line with these findings, significant cell death was observed in particular along the airways but also to a smaller extent in the lung parenchyma for the maximum exposure dose. Comparison to the in vivo data showed both, a good qualitative agreement regarding the site of the adverse effects and still more important a good quantitative agreement with the ex vivo obtained data. Using the ex-vivo model of the Isolated Perfused Rat Lung an in-vivo predictive estimate for respiratory toxicity from Nafamostat mesylate could be successfully derived.Further data are required to assess the predictive value of this model and the dosimetric concept and therefore its potential for reduction or refinement of in vivo testing for (sub-)acute inhalation toxicity.

Aerosol-related particle dosimetry: Novel approach for generation of complex aerosol atmospheres for nano-particle dose metric studies

Schwarz K, Ritter D, Koch W

Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

Knowledge on the relevant dose metric is crucial for the investigation and assessment of toxic effects in in-vitro and in-vivo models. Regarding theevaluation of the toxicity of inhaled nano-and ultrafine particles from combustion processes there is an ongoing discussion on the relevant dose metric. Depending on the physico-chemical properties, in particular aggregate state and surface properties, different metrics are proposed-particlemass, surface area ornumber.Investigationsinto the relevant dose metric require the generation of aerosol atmosphere over a wide particle size as well as massandnumberconcentration range without any changes in the composition of the aerosol and gas phase. The objective of the present works was to generate complex aerosol atmospheres forcell-basedin-vitro particle dose metric studies. Thepresent study aimed at the investigation of the biological effects of two strokeengines with exhaust fumes dominated by the condensed phasestemming from the lubrication oil.The variation of the number dose at constant mass dose is achieved by Brownian particle coagulation ageing of the pre-diluted exhaust gas within a laminar flow conditioning tube.The design of the conditioning unit allows for loss-free coagulation resulting in the increase of the particle size and reduction of the number concentration without any change,neitherin the mass concentration of the particle phase nor the concentration and composition of the gas phase. Variation of mass concentrationwas achieved by changing dilution and or exposure time. Deposition exhaust gas aerosol on the cell surface wasdetermined by adding nano-molybdenum oxide to the lubrication oil as a tracer. Since, in the exhaust gas, the molybdeneum nano-particles are incorporated in the oil droplets, the fraction of the oil droplets deposited on the cells equals the deposited fraction of the molybdeneum.In summary, we establisheda novel, uniqueapproach allowing for the generation of complex test atmospheres for nano-particle dose metric studiesfor exhaust particles of two-stroke engineswhere mass and number dose could be varied independently from each other. This allows for systematic nano-particle dose metric studiesof the biological/toxicologicaleffects of exhaust fumes.

Aerosol-related particle dosimetry: an experimental in vitro inhalation approachfor studying biological effects as a result of defined modification of particle number-andmass-based dose-metrics

Schwarz K, Koch, Ritter
Fraunhofer Institute for Toxicology and Experimental Medicine ITEM, Hannover, Germany

Defining the dose using a relevant dose-metric is of fundamental importance for evaluation and interpretation of toxicological data. Selection of the most appropriate dose-metric for inhalation exposure to aerosols is under discussion regarding specific physico-chemical properties of the test material such as particle size, but also types of the aerosols(e.g. poorly soluble, combustion, cigarette smoke).To enable experimental investigations on aerosol and dosimetry characteristics, an experimental in vitro inhalation model was established. It allowed for studying the otherwise unchanged toxicological properties of an aerosol in defined states regarding particle size, mass-and number concentration. Thus, the unique opportunity to investig atethe most relevant dose-metricfor aerosol exposure was realized.Afirst applicationused a highly concentrated aerosol of ultrafine particles from 2-stroke engine exhaust. An ageing unit based on Brownian coagulation allowedfor variation of number and mass concentration independently from each other. Acute local biological effects were investigated by exposure of air-lifted interface (ALI) human lung cultures (A549) under optimized exposure conditions (P.R.I.T.® ExpoCube®). Viability (WST-1), mitochondrial membrane potential (JC-1) and interleukin-8 release were analyzed.Dose relationships were studied after 30 or 60 minuteexposuresto four different aerosol conditions, each representing different number-and mean particle sizes atcomparable mass concentrations. Analytical determinationof particle depositionand filtered aerosol exposures validated discrimination between particle-and vapor-phase related effects. Strong correlations were found between particle mass dose and biological effects, but not between particle number dose and biological effects. It is expected that these characteristics are a consequence of the physico-chemical aerosol characteristics (oily) and therefore are in good agreement with classification of the relevant dose metrics which are under discussion for other aerosol types.Asignificant characterization ofcomplex relationships regarding dosimetry in inhalation by application of the in vitro inhalation modelis indicated by the results. Hence, it may be possible to get more insight and experimental evidence to find most appropriate dose metrics also for other types of aerosols.

Examination of the exposome in an animal model: the impact of high fat diet and rat strain on local and systemic immune markers following occupational welding fume exposure

KA Roach, V Kodali, M Shoeb, T Meighan, M Kashon, W McKinney, A Erdely, PC Zeidler-Erdely, JR Roberts, JM Antonini
NIOSH

In this study, an experimental model was designed to investigate the impact of multiple exposomal factors on susceptibility to acute lung inflammation following an occupationally-relevant pulmonary exposure, as well as immune responses involved in the subsequent resolution of inflammation. Genetic influence was addressed by using two different strains of rats (Sprague-Dawley [SD] and Brown Norway [BN]). To assess the potential impact of behavioral/lifestyle factors on the immune response, a high fat (HF) "Western diet" was also incorporated into the study. Male SD and BN rats were maintained on HF or regular (Reg) diets for 24wk, wherein inhalation exposure to stainless steel welding fume (WF) occurred between 7 and 12wk. A set of animals was euthanized at 7, 12, and 24wk to evaluate local and systemic immune markers corresponding to the baseline, exposure, and recovery phases of the study, respectively. At 7wk, animals maintained on the HF diet exhibited several notable alterations in immune markers independent of WF exposure—the effects of which were strain-dependent and more pronounced overall in SD rats. Rats fed HF exhibited increases in circulating leukocyte number and blood neutrophil proportionality, as well as elevated percentages of lymph node (LN) B-cells. At 12wk, indices of acute lung inflammation (LN cellularity, bronchoalveolar lavage [BAL] neutrophil number, alveolar macrophage activation) were elevated in all WF-exposed animals. Diet appeared to preferentially impact the SD strain at this time point again, as several inflammatory markers were further increased in HF animals over the Reg diet group directly following WF exposure. Comparatively, diet-associated effects in the BN strain were most evident at 24wk. Overall, the BN strain was slower to recover from WF exposure than SD rats, and this process was compromised further in BN rats fed the HF diet, as both local and systemic immune alterations were still evident in HF/WF BN rats at 24wk. Collectively, HF diet appeared to have a greater impact on immune status modulation and acute inflammatory responses in SD rats, but a more pronounced effect on inflammation resolution in BN rats. These results emphasize the importance of genetic, behavioral, and environmental factors in the modulation of immunological responsivity and highlight the often overlooked role of the exposome in shaping biological responses.

All animals used in this study were housed in the NIOSH Morgantown AAALAC-accredited animal facility. All experiments were performed in accordance with an ACUC-approved animal protocol (18-004) and all relevant guidelines and regulations set forth by CDC/NIOSH and AAALAC.

Physicochemical Drivers of Primary Toxicologic Outcomesinduced by Carbon Nanotubes and Nanofibers from U.S. Facilities

Fraser K[1,2], Yanamala N[3], Eye T[1], Friend S[1], Stefaniak A[1], Dahm MM[1], Schubauer-Berigan MK[4], Lersch T[5], Casuccio G[5], Bunker K[5], Hubbs AF[1], Kodali VK[1,2], Erdely A[1,2]
[1]NIOSH; [2]West Virginia University; [3]Rutgers Robert Wood Johnson Medical School; [4]IARC; [5]RJ Lee Group

Pulmonary exposure to carbon nanotubes or nanofibers (CNT/F) is known to induce inflammation, cytotoxicity, or tumorigenesis, and is a concern in the occupational setting. We have established toxicity profiles from male C57BL6/J mice aged8-10 weeks exposed to either 4 or 40 µg of one of nine different CNT/F via oropharyngeal aspiration as well as human epithelial BEAS-2B cells (0-24 µg/ml), differentiated THP-1cells (0-120 µg/ml), and human fibroblasts (0-2 µg/ml)for four primary outcomes: genotoxicity, inflammation, histopathology, and translocation. An overarching goal of our expansive study was to determine the relationship between particle physicochemical characteristics and those four major outcomes. The nine materials had a wide range of characteristics including diameter (6-397 nm), length (0.1-50 µm), surface area (18-238 m2/g), aspect ratio (2-1396),

residual metal catalyst (0.3-6.2 %), density (0.007-0.220 g/cm3). While all materials induced some extent of adverse outcomes, not all materials induced the same specific outcomes, or the same severity or persistence of quantified toxicity endpoints. The distinguishing physicochemical characteristics were noted as particle physical dimensions and agglomeration size and shape. As a general trend, materials of longer length and diameter, as well as particles with larger or less spherical agglomerations, were more likely to induce greater genotoxicity and more severe and persistent inflammation. Furthermore, the particle size and agglomeration characteristics were determinants of the severity and general location for histopathological changes, specifically the bronchial/bronchiolar or alveolar regions. Extrapulmonary translocation did not follow these same trends with a narrower range of peak liver accumulation at 84 days post-exposure which correlated with one day singlet lung burden. Importantly, physical dimension profiling indicated only a small population of individual CNT/F in the sample need to have the larger length and diameter to confer greater toxicity. The study identified physicochemical drivers of CNT/F toxicity, which was supported by an integrated approach, combining experimental evidence with computational modeling, and may have potential for broad application.

Comparison of Biological Response between Submerged, Pseudo-Air-Liquid Interface, and Air-Liquid Interface Exposure of A549 and Differentiated THP-1 Co-cultures to Combustion-Derived Particles

Kamaljeet Kaur[1], Raziye Mohammadpour[2,4, 7], Anne Sturrock[3], Hamidreza Ghandehari[2,4,5], Christopher Reilly[2,6], Robert Paine III[3], and Kerry E. Kelly[1,2*]

[1]Department of Chemical Engineering, University of Utah, Utah, USA; [2]Utah Center for Nanomedicine, University of Utah, Utah, USA ; [3]Division of Pulmonary and Critical Care Medicine, University of Utah, Utah, USA ; [4]Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Utah, USA ; [5]Department of Biomedical Engineering, University of Utah, Utah, USA; [6]Department of Pharmacology and Toxicology and Center for Human Toxicology, University of Utah, Utah, USA; [7]mRNA Center of Excellence, Sanofi, Waltham, MA, USA.

Air liquid interface (ALI) exposure systems are gaining interest, and studies suggest enhanced response of lung cells exposed to particles at ALI as compared to submerged exposure, although the results have been somewhat inconsistent. The majority of ALI studies have used monocultures and measured particle deposition using assumptions including consistent particle deposition, particle density, and shape. This study exposed co-cultures of A549 and differentiated THP-1 cells to flame-generated particles using three exposure methods: ALI, pseudo-ALI, and submerged. The dose at ALI was measured directly, reducing the need for assumptions about particle properties and deposition. For all exposure methods an enhanced pro-inflammatory response (TNFα) and Cytochrome P450 (CYP1A1) gene expression, compared to their corresponding negative controls, was observed. ALI exposure induced a significantly greater TNFα response compared to submerged exposure. The submerged exposures exhibited greater induction of CYP1A1 than other exposure methods, although not statistically significant. Some of the factors behind the observed difference in responses for the three exposure methods include differences in physicochemical properties of particles in suspending media, delivered dose, and potential contribution of gas-phase species to cellular response in ALI exposure. However, given the difficulty and expense of ALI exposures, submerged exposure may still provide relevant information for particulate exposures.

New Approach Methodologies for the hazard assessment of nanocellulose (NC): Tier 1 Testing – High Content Analysis Based Evaluation of the Toxicity of NC Materials in Human Intestinal and Macrophage Models

Kevin Hogeveen[1], Anne-Louise Blier[1], Jan Mast[2], Eveline Verleysen[2], Olimpia Vincentini[3], Francesco Cubadda[3], Ludovic LeHegarat[1], Valerie Fessard[1]

[1]French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Fougères, France; [2]Sciensano, Brussels, Belgium; [3]Istituto Superiore di Sanità -National Institute of Health, Rome, Italy

Nanocellulose (NC) is an emerging material in the food sector with foreseen applications in food packaging, novel food, as well as food additives. To date, the potential hazards associated with oral exposure to NC are not well characterized. The nanoscale features of NC require a nano-specific assessment, which should focus on whether cellulose nanofibers may display local toxicity and if they are able to cross the intestinal epithelia, leading to systemic exposure. A tiered approach was implemented for the hazard evaluation of a panel of NC samples including nanofibrillated cellulose (NFC), cellulose nanocrystals (CNC) and bacterial nanocellulose (BNC) materials.

In the first tier, a high content analysis-based approach was used to obtain a maximum amount of information on the cellular responses in intestinal and macrophage cell models following exposure to a panel NFC, CNC and BNC materials. A series of endpoints including cytotoxicity, DNA damage response, oxidative stress, and the pro-inflammatory response was quantified following a 24h treatment of Caco-2 and THP-1 cells with NC. No cytotoxic effects on the panel of endpoints were observed in the intestinal Caco-2 model. However, in differentiated THP-1 cells, while only slight cytotoxic effects were observed on cell counts and nuclear morphology, significant increases in pro-inflammatory responses (IL-8 secretion) were observed.

A selection of NC materials demonstrating significant cytotoxic effects in Tier 1 studies will be further investigated in Tier 2 studies where the uptake and crossing of the intestinal barrier will be assessed. Tier 2 will also involve the assessment of local effects, including inflammation and genotoxicity, of NC on complex models of the gastrointestinal epithelium. As well, effects of digestion and modification by the human microbiome will be studied. Tier 3 testing will involve repeated dose toxicity assessment in complex models of the intestinal epithelium.

Inflammation and transcriptome changes induced by asbestiform fibers in mice are ameliorated by a small molecule synthetic

Kinta M. Serve[1], Wesley Wilson[2], Reagan Badger[1], and Melpo Christofidou-Solomidou[2]

[1]Idaho State University; [2]University of Pennsylvania

Asbestos exposure leads to the development of chronic, life-threatening diseases like malignant mesothelioma, pleural disease, and asbestosis. Due to the long disease latency, asbestos-related disorders are often not detected until they reach a chronic, often fatal stage, thus limiting effective therapeutic options. It is thought that unresolved inflammation following asbestos exposure contributes to disease development. As such, early intervention with anti-inflammatory molecules may prevent disease progression to a chronic state. The flaxseed lignan secoisolariciresinol diglucoside (SDG) is a small molecule antioxidant and free radical scavenger, with anti-inflammatory effects in various disease models. Here, we describe the in vivo immune and gene regulating activity of a synthetic SDG during

the acute inflammatory stage following asbestos exposure. All animal experiments were approved by an IACUC committee and overseen by a staff veterinarian. Male and female C57BL/6 mice were administered daily LGM2605 (100mg/kg) via gel cups for 3 days before and 14 days after 200µg Libby amphibole asbestos (LAA) given i.p. Control mice were given unsupplemented gel cups and an equivalent dose of i.p. saline. On day 14 post-LAA treatment, peritoneal lavage was assessed for immune cell influx, cytokine concentrations, oxidative stress biomarkers, and immunoglobulins. RNA was extracted from peritoneal tissue and transcriptomics analyzed. Our data demonstrate altered trafficking of both innate and adaptive immune cells, increased pro-inflammatory cytokine concentrations, induction of immunoglobulin isotype switching, and increased oxidized guanine species. LGM2605 countered these changes similarly among male and female mice, ameliorating late inflammation and altering immune responses in late post-LA exposure. RNAseq data comparing gene changes in mice exposed to LAA vs. control revealed differential expression in genes associated with neoplasm metastasis and DNA repair pathways. Of particular interest, genes involved in the PTTG1 pathway of tumorigenesis were significantly affected by LAA. These gene changes were mitigated by treatment with synthetic SDG. Together, these data support the efficacy of SDG as a chemopreventative agent of asbestos related disorders. These data also suggest possible efficacy of SDG in treatment of inflammatory conditions.

Identification of potential mediators implicated in the carcinogenicity of poorly soluble low toxicity particles in rats

Laeticia Perez
Université Catholique de Louvain (UCLouvain), Belgium

Background: Poorly soluble low toxicity (PSLT) particles, e.g. titanium dioxide and carbon black, can induce chronic inflammation and lung cancer in rats at exposure levels leading to lung particle overload. Overload is observed at very high exposure doses and is associated with impaired alveolar macrophage function and mobility. Although overload of PSLT has been observed in several experimental species (rats, mice, hamsters, monkeys), the rat appears as the only one that develops chronic inflammatory and carcinogenic lung responses. Whether these responses can be expected or not in humans exposed to PSLT particles remains a source of debate and has led to the classification of PSLT as possible carcinogens. In order to clarify human situation, it is important to understand the exact cellular events leading to adverse lung outcomes in rats. This study was thus designed to identify possible mediators and pathways of PSLT carcinogenicity in rats by comparing the responses of rat and mouse alveolar macrophages under overload conditions.
Methods: To avoid animal testing, naïve alveolar macrophages from rats and mice were exposed in vitro to titanium dioxide or carbon black particles at doses leading to non-overload or overload conditions. Four days after exposure, transcriptomic analyses were performed to assess and compare gene expression in the different conditions and species.
Results: Regarding mouse macrophages, there were no significantly differentially expressed genes at overload conditions of either titanium dioxide or carbon black particles. In contrast, a total of 128 and 101 genes were found to be differentially expressed in rat alveolar macrophages exposed to titanium dioxide or carbon black particle overload respectively. Among these genes, 20 were common to both PSLT particles. Seven of these 20 genes were identified as potential key mediators as they were related to inflammation or cancer development. The other genes were for the most part linked to overload.
Conclusion: This study identified 7 potential mediators that may be involved in the development of chronic inflammatory and carcinogenic lung responses in rats exposed to very high doses of PSLT particles.

Dietary Supplementation with Omega-Fatty Acid Prevents Silica-Triggered Autoimmune Disease in Adult Lupus-Prone Mice

Lauren K. Heine, Tasha Scarlett, James G. Wagner, Ryan P. Lewandowski, James J. Pestka, and Jack R. Harkema
Michigan State University, East Lansing, MI,USA

Lupus is an autoimmune disease that affects multiple organ systems. Inhalation exposure to crystalline silica dust (cSiO2) has been epidemiologically linked to disease manifestation. Previously, we have shown that dietary supplementation with the omega-3 fatty acid, docosahexaenoic acid (DHA), prevents cSiO2-triggered autoimmunity and pathology in the lungs and kidneys of juvenile, 8-week-old, lupus-prone mice. In the present study, we tested the hypothesis that dietary supplementation with DHA at a human dose equivalent of 5 g/d prevents inhaled cSiO2-triggered autoimmune disease in adult lupus-prone mice that more accurately reflect the age of cSiO2-exposed workers. Female 14-week-old NZBWF1 mice were started on either a purified AIN-93G control (CON) or AIN-93G DHA amended diets. Two weeks later, mice were intranasally instilled with either saline vehicle (VEH) or 1 mg cSiO2 once per week for 4 consecutive weeks and sacrificed at 1 or 5 weeks after the last instillation (PI). Lung and kidney tissues were processed for light microscopy, immunohistochemistry, and morphometric analysis. Bronchoalveolar lavage fluid (BALF) was collected for total and differential inflammatory cell counts. VEH/CON mice had no pulmonary or renal pathology at either 1- or 5-weeks PI. cSiO2/CON mice had mild pulmonary ectopic lymphoid tissue (ELT) formation at 1-week PI, with a marked increase at 5-weeks PI. Correspondingly, lungs of cSiO2/CON mice had increases in CD3+ T-cell, CD45R+ B-cell, and IgG+ plasma cell densities at both timepoints compared to VEH/CON mice. Significant cSiO2-induced increases in macrophages and neutrophils were also found in BALF. Kidneys from cSiO2/CON mice had conspicuous glomerular IgG deposition that was absent in VEH/CON mice. Dietary DHA supplementation dramatically attenuated cSiO2-triggered lung pathology including ELT formation, increases in CD3+T-cell, CD45R+B-cell and IgG+ plasma cell densities, and inflammatory cell counts in BALF at both 1- and 5-weeks PI. cSiO2/DHA mice also had no IgG deposition in renal glomeruli. These results demonstrate that dietary DHA markedly prevents cSiO2-triggered lupus pathology in adult mice, in a manner similar to juvenile mice of the same sex and strain.
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QCL-Based Spectroscopy for Rapid Identification of Microplastics

Louis Tisinger
Application Scientist, Molecular Spectroscopy Agilent Technologies

Recognition of microplastics as a significant environmental pollutant is a fairly recent development, and the means by which

to analyze them are quickly developing. Larger plastic fragments, those greater than a millimeter, can be easily interrogated by physical and spectroscopic methods, e.g., visible microscopy, density, melting point, macro-infrared spectroscopy, etc. The latter, infrared spectroscopy, is a very good way to analyze particles, owing to the specificity of IR spectra; an IR spectrum is a molecular "fingerprint". Microplastic particles, i.e., those that are less than a millimeter in diameter, require more complex methods. In order to measure them, they need to be found in a field of view, and then, using an appropriate spectrometric method, be measured/identified. Fortunately, there are now available technologies, including automated single-point infrared microscopes and imaging systems - both linear array and focal plane array - which streamline the process of collecting particle spectra. However, identification, via library searching, tends to be a distinct step in the MP workflow of these systems. Furthermore, utilizing infrared microscopes and imaging systems typically requires significant technical knowledge. To that end, a new system has emerged which automates the entire microplastics analysis workflow: a quantum cascade laser (QCL)-based analyzer. This presentation will (i) show details on the operation of the system (ii) describe the simple microplastics analysis workflow, and (iii) present some sample data.

Nearly Free Surface Silanols Dictate Membrane Disruption That is Attenuated by Cholesterol Content

Matthew J. Sydor[a], Rebekah L. Kendall[a], Cristina Pavan[bcd], Francesco Turci[bce], and Andrij Holian[a]
[a]Centerfor Environmental Health Sciences, Department of Biomedical and Pharmaceutical Sciences, University of Montana, USA. [b]Department of Chemistry, University of Turin, Italy [c]G. Scansetti" Interdepartmental Centre for Studies on Asbestos and Other Toxic Particulates, University of Turin, Italy [d]Louvain centre for Toxicology and Applied Pharmacology, Université catholique de Louvain, Belgium [e]Nanostructured Interfaces and Surfaces Interdepartmental Centre, University of Turin, Italy

Inhalation of crystalline silica has been linked to chronic lung diseases such as silicosis. Respirable silica particles can deposit into the alveolar spaces of the lungs and be encountered by alveolar macrophages, which phagocytose the material. Silica has been demonstrated to have membranolytic properties and cause an event known as phagolysosomal membrane permeability (LMP), which is a key step leading to NLRP3 inflammasome formation and subsequent IL-1b and IL-18 release. Thus, LMP is a key step in the overall inflammatory pathway caused by silica. In this work, we studied mechanisms of how silica may cause LMP by disrupting model lipid membranes. Time-resolved fluorescence anisotropy measurements of the membrane dye, Di-4-ANEPPDHQ, in 100 or 500-nm liposomes were used to determine changes to lipid order caused by five different silica materials. A decrease (increase in lipid order) to the wobble-in-a-cone angle of Di-4-ANEPPDHQ in 100 and 500-nm liposomes composed of phosphatidyl choline (PC), were dose-dependent on nearly free surface silanols (NFS) of the silicas. Treatment of 100-nm phosphatidyl serine (PS) liposomes resulted in no change toTo do this, standard mouse chow and Nicotinamide Mononucleotide (NMN) chow was pulverized using a mortar and pestle and then sieved with a 40 µm sieve. The sieved chow is membrane order. Addition of cholesterol to 100-nm DOPC liposomes attenuated the increase in lipid order caused by the silica materials. NFS containing silicas caused a dose dependent increase in hemolysis of red blood cells (RBC) isolated from sheep blood. Incubation of the silica materials with PC micelles prior to the hemolysis assay reduced silica-induced hemolysis. This same effect did not occur when incubating with PS micelles, demonstrating a preferential interaction between NFS on the silica surface and PC. These results demonstrate a potential phospholipid target of silica when LMP occurs and membrane disruption that could lead to lysis. NFS seem to promote this interaction between silica and lipid membranes and subsequent membrane disruption could be regulated by membrane cholesterol.

Identification of Laboratory Animal Microplastic Consumption: A Quantitative Approach

Rachel Templeton [1,2], Marcus Garcia[2], Rui Liu[2], David Scieszka[2], Russel Hunter[2], Eliseo Castillo[3], Matthew Campen[2]
[1]University of Pittsburgh; [2]University of New Mexico: Department of Pharmaceutical Sciences; [3]University of New Mexico, School of Medicine, Division of Gastroenterology and Hepatology

Worldwide, 7 billion tons of plastic have been produced with most plastics never decomposing. Instead, these plastics break down, forming microplastics (MPs) that are now prevalent throughout the environment. Human exposure to MPs through inhalation, ingestion and absorption is well established, with the average person consuming 5g of microplastics per week. However, identifying and quantifying microplastics in biological organisms is still under investigation. The Campen Lab identified that 5um polystyrene microspheres translocate from the GI tract through systemic circulation into the brain, liver, kidneys, and possibly to the lungs. In researching MP's consumption, it is essential to understand and control the exposure routes that are not under investigation. This study is focused on identifying and quantifying plastic exposure via ingestion by studying laboratory mouse chow. To do this, standard mouse chow and Nicotinamide Mononucleotide (NMN) chow was pulverized using a mortar and pestle and then sieved with a 40 µm sieve. The sieved chow is then digested with 10% KOH in the incubator at 40oC with agitation for 72 hours to separate MPs from the organic components of the chow. The samples are then ultracentrifuged at 30,000g for 4 hours, and supernatant is removed to isolate the MPs in the sample. The samples were re-suspended in EtOH and stored for imaging. MPs in both chow groups were identified via light microscopy. Isolated MPs were then degraded in 100% Acetone, and pyrolysis Gas chromatography/Mass Spectrometry (Py-GC-MS) was performed. Through this process, Py-GC-MS breaks down the sample into metabolites and plasticizers that can be used as a fingerprint to quantitate MPs in the chow. This research will aid in identifying MP's consumption via chow in lab mice. This will provide insight into ingested MPs exposure in existing and future research. MPs are unavoidable due to universal exposure, and the evolution of disease-associated is still largely unexplored. This study is focused on aiding in developing techniques to identify, isolate, and quantify MPs to aid in the early identification of poor health outcomes associated with MP exposure.

Toxicological impact of Secondary Organic Aerosol compounds in air-liquid-interface exposed lung cell models

S. Offer[1], E. Hartner[1], T.Gröger[1], Y. Rudich[2], M. Prado[2], A. Kiendler-Scharr[3], T. Hohaus[3], A.Paul[3], S. Di Bucchianico1, M. Sklorz[1], H. Czech[1], O. Sippula[4], P.Jalava[4], T. Adam[5] and R. Zimmermann[1] & all aeroHEALTH consortium members
[1]Helmholtz Zentrum München & University of Rostock, Germany [2]Weizmann Institute, Israel [3]Forschungszentrum Jülich GmbH, Germany [4]University of Eastern Finland,Finland [5]Universität der Bundeswehr München, Germany

In the framework of the aeroHEALTH Helmholtz International Lab(www.aeroHEALTH.eu),the impact of atmospheric aging on aerosol particle-induced health effects is elucidated. A model experiment addresses differences in toxicological effects of Secondary Organic Aerosols (SOA)generated by photochemically aging of either a biogenic (β-pinene) or an anthropogenic (naphthalene) gaseous organic precursor(seed: soot particles, SP). Two different lung cell models were exposed to the model aerosols at the air-liquid interface (ALI).Lung epithelial cells(A549) and a co-culture model(A549/EA.hy926-endothelial cells) were exposed for 4 h to different aerosol concentrations of pure SP,β-pinene-SOA(SOAβPIN-SP) or naphthalene-SOA(SOANAP-SP).The aerosols were comprehensively physico-chemically characterized and cytotoxicity, intracellular oxidative stress, genotoxicity, inflammatory effects etc. were determined(Offer et al., EHP 2022)followed by a RNAseq transcriptome analysis. Both SOA-types caused significant toxicological effects with greater adverse impact of SOANAP-SP compared to SOAβPIN-SP. At functional level, SOANAP-SP augments e.g. secretion of malondialdehyde (lipidperoxidation) and IL-8. An activation of the endothelial cells (co-culture) was confirmed by comet assay, suggesting secondary genotoxicity. Chemical characterization of PM revealed distinct differences in the composition of the two SOA-types. It is shown that SOA-compounds can increase the toxicity of primary SP. Aromatic precursors, such as naphthalene, form more oxidized and aromatic SOA of higher oxidation potential with higher toxicity compared to a liphatic precursors(e.g.β-pinene).The influence of atmospheric chemistry on the chemical PM composition thus play a crucial role for the adverse health-out comes of emissions. Recently fresh and photochemical aged gasoline car emissions (EURO 6) were tested, showing a significant toxification of the emissions by the photochemical aging processes. This highlights the importance of aging and SOA for the health effects of atmospheric particles.

Which chemical aerosol properties shall be measured in particle toxicological studies? Novel approaches for particle characterisation to complement aerosol toxicological studies

Johannes Passig[1,2], Robert Irsig[3], Hendryk Czech[1,2], Patrick Martens[1,2], Julian Schade[1], Sven Ehlert[3] and Ralf Zimmermann[1,2]
[1]Analytical Chemistry, University of Rostock, Rostock, Germany; [2]CMA, Helmholtz Centre Munich, Munich, Germany; [3]Photonion GmbH, Schwerin, Germany

The air pollution with inhalable particle matter (PM) represents the most severe environmental health-risk world wide. The chemical composition of the particles is considered to be highly relevant for the PM-toxicity. In particular, the content of toxic compounds such as soot, polycyclic aromatic hydrocarbons (PAHs) or transition metals (e.g. Fe or Cu) is of concern. In conjunction with particle toxicological studies (e.g. with Air Liquid Interface (ALI) lung cell model exposures or animal inhalation experiments), usually a detailed analysis of the PM composition is performed by chemical analysis of filter samples. This gives integral values on the pollutant concentrations (i.e. amount per cubic meter).However, any information on the mixing state of toxicants is missing. The mixing state, however, likely is crucial to assess health effects: the toxicants may either be equally distributed over many particles (internally mixed) or could be highly concentrated within a specific, small sub-population (externally mixed), inducing different effects upon particle in-lung deposition. In the latter case, the particles with a very high concentration of toxicants can induce stronger cellular effects at the deposition site due to overwhelming defense mechanisms locally (e.g. antioxidant capacity depletion) or due to DAMP signaling, if necrotic cell death is induced. Consequently, novel on-line analysis techniques, addressing the mixing state of health relevant PM-chemicals on a single-particle scale are required. A new approach for on-line single particle analysis bases on bipolar laser mass spectrometry. The aerosol is directly sampled from the air. The organic coating of the size-classified aerosol particles (laser velocimetry) is desorbed by an IR-laser pulse on the fly. A few µs later, the relevant toxicants(transition metals, PAH and soot) are ionized by a novel combined laser ionization scheme and are detected in the mass spectrometer. The new method has been applied in conjunction with ALI exposure toxicological studies e.g. for combustion emission monitoring. It gave insight into the mixing state of the air toxicants (PAH/metals/soot)and other compounds (e.g. nitrate, sulfate etc.)

Evaluation of WS1442 on Systemic Inflammation and Blood Brain Barrier

Raul Alejandro Salazar, Russell Hunter, Selita Lucas, David Sciezka, Guy Herbert, Matthew Campen, Barry Bleske
Department of Pharmaceutical Sciences, The University of New Mexico-Health Sciences Center

Exposure to adverse environmental stressors, such as ozone, can induce cellular reactive oxygen species and may increase the risk of developing chronic diseases, inflammation. Furthermore, systemic inflammation is a key process in the development and progression of many comorbid states including cardiovascular, pulmonary, and neurological diseases. An important therapeutic endpoint is reducing oxidative stress and inflammation. WS 1442, an extract of hawthorn leaves and flowers has been shown to improve endothelial function, cardiac contractility, and decrease oxidative stress. However, the effect of WS 1442 in reducing toxicant-driven systemic and neuroinflammation is not well characterized. Given the WS 1442 effects on decreasing oxidative stress and improving endothelial function, it is likely that WS 1442 will have benefit regarding systemic inflammation, thereby indirectly improving certain cardiovascular, pulmonary, and neurological conditions. Thus, our overall hypothesis is that treatment with WS 1442 will ameliorate pulmonary and neuroinflammatory effects (secondary to compromised blood brain barrier integrity) caused by ozone inhalation. We treated C57BL/6 mice with WS 1442 at 150 mg/kg. Administration of WS 1442 occurred for 2 weeks every-other-day via gavage, then switched to a 1.5-week daily gavage prior to ozone exposure. On the last day of administration, mice were exposed to ozone for 4-hour at 1ppm, specifically 24 hours prior to euthanasia. Assessment of localized mRNA expression levels via qPCR in lungs and frontal cortex was used to evaluate the inflammatory response. Lungs demonstrated a significant increase in inflammatory cytokine expression (interleukin-6) that was completely abrogated by WS 1442 treatment. Results indicates that WS 1442 lowers indicators of neuroinflammation, but ozone exhibited minimal observable impact on the brain. Overall, this study model and preliminary results will allow us to better understand and assess the potential for WS 1442 to reduce the adverse effects of environmental stressors.

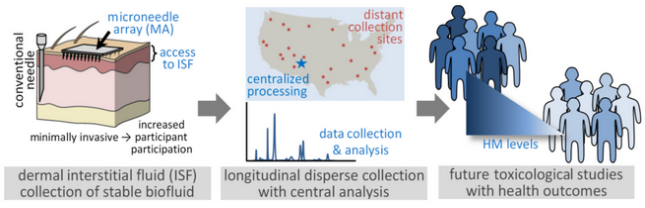
The effects of photochemical aging and interactions with secondary organic aerosols on cellular toxicity of combustion particles

Reuben Attah, Kamaljeet Kaur, Kerry Kelly
Department of Chemical Engineering, University of Utah, United States

Fine particulate matter (PM 2.5) is associated with numerous adverse health effects, such as pulmonary and cardiovascular diseases and premature death. Primary combustion particles and secondary organic aerosol (SOA)derived from the oxidation of anthropogenic VOCs are significant components of ambient particulate matter. Atmospheric aging induces changes in their chemical composition. However, the impact of these changes on human health is poorly understood. This study aims to understand how the atmospheric aging of primary combustion particles and its interaction with secondary organic aerosols affects biological responses. Fresh combustion particles were produced by combusting a jet-fuel surrogate in the flat-flame burner and photochemically oxidized in a potential aerosol mass (PAM)oxidation flow reactor. SOA was produced by the oxidation of toluene vapor in the PAM reactor. The PAM simulated atmospheric aging of 10 days by exposing the combustion particles and combustion particles coated with SOA to OH radicals in the presence of 245nm ultraviolet light, humidity, and ozone.TheO3/OH andHO2/OH ratios in the PAM reactor were similar to atmospheric values. The particle size distribution, molecular structure, and composition of the soot particles were characterized by SMPS, FTIR, and GCMS, respectively. The GC mass spectra revealed that the PAH of fresh soot particles was significantly higher than aged soot particles because most of the PAHs are converted to oxy-PAH because of oxidation to aged soot particles. Monocultures of human epithelial cellsA549 and co-cultures of A549 and THP-1 macrophages grown under submerged conditions were exposed to the three different particle types at concentrations of 12ug/cm2 6ug/cm2 and 1.5ug/cm2and subsequently evaluated for cytotoxicity, xenobiotic metabolism(CYP 1A1), and pro-inflammatory biomarkers, IL-8 and TNF-alpha. Preliminary results suggest that combustion particles with SOA exhibited the greatest toxicity and fresh combustion particles exhibited the greatest CYP 1A1 response.

Quantifying Heavy Metals in Interstitial Fluid

Robert M. Taylor, PhD[1], Yiliang Zhu[2],AliciaBolt, PhD[3], Justin T. Baca, MD, PhD[1]
[1]University of New Mexico, Department of Emergency Medicine; [2]University of New Mexico, Department of Internal Medicine; [3]University of New Mexico, Department of Pharmaceutical Sciences



Chronic exposure to heavy metals (HM) is associated with many detrimental health effects. HM contamination in soil and water costs trillions of dollars annually to the U.S. and global economies. HM contamination particularly relevant to human health in the Western U.S. includes Arsenic (As), Cadmium (Cd), Uranium (U), and Vanadium (V). Toxicity can be attributed to individual HMs, however exposure to multiple HMs is suspected to have additive or synergistic harmful health effects. Our hypothesis is that microneedle array (MA) extraction of interstitial fluid (ISF) will enable minimally invasive quantitation of heavy metal (HM) exposure. We aim to establish analytical parameters for ICP-MS analysis of HMs, quantify baseline HM content in ISF vs other fluids, and characterize a mixed HM exposure model. We ultimately envision a wearable microneedle patch that could be mailed to individuals or distributed through community centers, worn for a few hours, and returned to a central laboratory. ISF can be collected with MAs and is a rich source of disease and exposure biomarkers. Recent advances in ISF extraction suggest a minimally invasive method that can be adapted to monitor exposure and biological loads longitudinally. We recruited healthy human volunteers under a protocol approved by the UNM Human Research and Resource Committee. Subjects had ISF, collected with MAs, blood, and urine collected. Additionally, under a protocol approved by the UNM animal care and use program, 36 Sprague Dawley rats were unexposed (n=6), controls (n=6) (exposed to ad libitum water containing a mixture of As, Cd, U, and V, each at 5X the maximum contaminant level (MCL) for drinking water), or in high As (n=6), high Cd (n=6), high U (n=6), or high V (n=6) cohorts with either As, Cd, U, or V, respectively, increased to 50X the MCL. ISF, collected with MAs, and blood were collected for 8 weeks. Metals were then quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Our preliminary results suggest similar HM concentrations in ISF, compared with blood, in small unexposed human and animal populations. All four metals can be successfully quantified in tandem using ICP-MS, metal burden can be compared between the cohorts, and correlations between the different metals is currently being examined.

Dietary Modulation of Pulmonary Lipids Influencing Toxicological Responses to Ozone Inhalation in Mice

Russell Hunter[1], David Sciezska[1], Brenna Baird[1], Marcus Garcia[1], Jessica Begay[1], Selita Lucas[1], Guy Herbert[1], Haiwei Gu[2], Barry Bleske[1], Matthew Campen[1]
[1]University of New Mexico, Department of Pharmaceutical Sciences; [2]Florida International University, Center for Translational Science

Lipid and protein composition of the epithelial lining fluid of the lung facilitate interactions with xenobiotic agents and the degree of inflammatory reactions within the alveolar space. As a source of oxidative damage, the inhalation of ozone is a known driver of inflammation in the lung that leads to systemic release of biological mediators into the circulatory system effecting vascular tone. The current study seeks to examine how dietary supplementation of saturated and polyunsaturated fatty acids effect the overall lipidomic profile of the lung, as well as how these changes modulate the biological effect of ozone inhalation on the lungs. The experimental design utilized female C57BL/6 mice as a model, with animals receiving diets containing soybean, flaxseed, or coconut oil. Animals were then exposed to either 1PPM ozone, or filtered air for 4 hours. 24 hours post exposure, animals were evaluated for pulmonary inflammation via bronchoalveolar lavage (BAL), and lungs were taken for lipidomic analysis. Cell counts for the BAL showed increased inflammation after ozone inhalation, with a pronounced increased in inflammation in the coconut oil fed animals when compared to other dietary groups. Lipidomic analysis of the lungs identified 373 lipid species differently expressed based off of diet, as well as 135 lipid species differentially altered by exposure. Notably, PIP2(20:2) was shown to positively correlated with ozone exposure, and PIP2 has canonically been shown to be a critical

regulator of vascular ion channels. Overall, the coconut oil diet seemed to alter the lipidomic profile of the lung in a manner that increased inflammation. This study highlights how dietary supplementation of fatty acids can have large effects on organ systems beyond the digestive tract, and provides clues for potential dietary intervention strategies to limit risks to human health due to air pollution induced pulmonary damage.

Particulate Toxicology in the 21st Century: Organotypic Models Reveal Trans-Epithelial Effects of Particulates in the Respiratory Tract

Shaun D. McCullough
Principal Investigator, US Environmental Protection Agency, Chapel Hill, North Carolina, USA

Complex multi-cellular in vitro models of the human respiratory tract have provided novel opportunities to advance our understanding of the effects of inhaled particulate exposures on human health. These "organotypic" cell culture models also provide new opportunities to evaluate the impact of inter-individual variability and susceptibility/vulnerability factors on exposure outcomes, and the potential to increasing both the physiological relevance and throughput of particulate toxicology research and testing. This presentation will provide a brief introduction to organotypic multi-cellular in vitro models, examples of how we have used them to characterize the effects of model particulate exposures, and critical considerations for their future use in particulate toxicology. Specifically, data will be presented demonstrating that exposure of a bronchial epithelial barrier to diesel exhaust particulates causes redox imbalance, NRF2 activation, and oxidative stress in underlying lung fibroblasts (i.e., "trans-epithelial" effects) in a multi-cellular model of the tracheobronchial epithelium. The presentation will also describe our development of a novel tri-culture model of the alveolar-microvascular interface and the subsequent demonstration that exposure of an alveolar epithelial barrier induces trans-alveolar oxidative stress and interleukin (IL)-8 release in microvascular endothelial cells. Data will also be presented demonstrating that NRF2-dependent IL-8 secretion from the lung microvascular endothelium is dependent on the activation of mitogen activated protein (MAP) kinases in the adjacent alveolar epithelial barrier. Finally, data regarding the inter-individual variability in in vivo-relevant in vitro assay endpoints and the effect of dosing air-liquid interface differentiated cells by liquid application on common toxicological endpoints and global transcriptional programming will be discussed in the context of the application of organotypic in vitro models in particulate toxicology. Overall, this presentation will provide key examples of how organotypic models of the human respiratory tract can be used to revolutionize our understanding of the cellular and molecular mediators of particulate-induced health effects while also providing a high(er) throughput platform for inhaled particulate screening, testing, and risk assessment.

Particulate Air Pollution and Viral Activation by Airway Proteases: Assessed by a Novel Assay in Human Nasal Epithelial Cells

Stephanie A Brocke[1], Yongho Kim[2,3], M. Ian Gilmour[2], Weidong Wu[4], Ilona Jaspers[1,3]
[1]Curriculum in Toxicology and Environmental Medicine, University of North Carolina, Chapel Hill, NC, USA; [2]Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA; [3]Center for Environmental Medicine, Asthma, and Lung Biology, University of North Carolina, Chapel Hill, NC, USA; [4]School of Public Health, Xinxiang Medical University, Xinxiang, Henan Province, China

Several epidemiological studies have linked ambient particulate matter with increased respiratory viral infection severity and mortality. Previous in vitro studies demonstrated causality between particulate exposure and enhanced susceptibility to viral infection, though the mechanisms driving this interaction remain unclear. During respiratory viral infection, viruses such as Influenza H1N1 and SARS-CoV-2 must be activated by endogenous proteases expressed by the host. In humans, transmembrane protease serine S1 member 2 (TMPRSS2), human airway trypsin-like protease (HAT), and Furin, among others, are known to activate a variety of respiratory viruses. The activity of these proteases is naturally throttled by the expression of antiproteases in the airway such as secretory leukocyte protease inhibitor (SLPI). Previously, we have shown that exposure to ozone enhances proteolytic activation of influenza by upsetting the protease and antiprotease balance in the airway. Using a novel assay with internally quenched fluorescent peptides mimicking the cleavage site of viral fusion proteins, we have demonstrated apical proteolytic activity in cultures of differentiated human nasal epithelial cells (hNECs). We investigated whether exposure to air pollution particulates from various sources would impact apical proteolytic activity. Woodsmoke particles derived from red oak resulted in enhanced cleavage of the SARS-CoV-2 Spike peptide at 24 h post exposure, though did not impact cleavage of influenza hemagglutinin. We next assessed whether particulate exposure altered expression patterns of prominent proteases and antiproteases involved in viral activation. Together, these findings suggest a possible mechanism by which exposure to particulate air pollution can enhance susceptibility to respiratory viral infection and this assay demonstrates utility for screening air pollutants for their ability to enhance apical proteolytic activity.

Inhaled Polyamide Particles Reduce Uterine Vascular Reactivity in Virgin Rats

TN Seymore[1], CM Cary[1], D Singh[2,3], P Demokritou[2,3], PA Stapleton[1,2]
[1]Department of Pharmacology and Toxicology, Rutgers University, [2]Nano-Biosciences & Advanced Materials Center, Rutgers University, [3]EOHSI, Rutgers University, Piscataway, NJ 08854

Particulate matter is a huge contributor to the risk of cardiovascular events. Specifically, there is evidence that particulate matter compromises vascular function by disrupting endothelial function. A common constituent of indoor particulate matter are micro- and nanoplastics (MNP). To our knowledge, the toxicological consequences on the cardiovascular system of in vivo exposure to MNP via inhalation are unknown. Utilizing polyamide-12 (i.e., nylon; Palmer-Holland) as a representative MNP, the objective of this study was to determine if MNP inhalation would elicit vascular dysfunction. Virgin female Sprague Dawley rats in estrus were exposed to MNP aerosols [10.3 ± 0.1 mg/m3; geo. mean particle size 398.34 ± 1.78 nm (SMPS, TSI); aerodynamic diameter 3.0 ± 1 µm (APS, TSI)] via whole-body inhalation (HPGA, IESTechno) for 4-5 hours. Characterization indicates aerosolized particulate is well within the MNP definition. 24 hours after exposure, reactivity of the macrocirculation (aorta and uterine artery; DMT, Ann Arbor, MI) and the microcirculation (LSI, St. Albans, VT) were evaluated using wire or pressure myography, respectively. All animal experiments had Rutgers IACUC approval. Responsiveness to increased concentrations of vasoactive chemicals [methacholine (MCH), sodium nitroprusside (SNP), and phenylephrine (PE); 10-9M to 10-4M] was recorded for each technique. Results showed significantly reduced endothelial-dependent vessel reactivity

after MNP exposure in both the uterine and radial arteries after MCH administration. Vascular smooth muscle responses were also significantly impaired in the radial artery, demonstrating a greater propensity for vascular contraction compared to control (-9.56 ± 7.28% vs -59.7 ± 17.4%). There were no significant changes in aortic responses. This data demonstrates effective MNP aerosolization and exposure and shows that inhalation of plastic particles can lead to vascular impairments. The mechanisms involved in impaired vascular function remain unknown; therefore, the next steps are to investigate the pathways for vascular reactivity in both the endothelium and smooth muscle.

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Diesel Exhaust Particle Exposure Induces Polarization-State Dependent Functional and Transcriptional Changes in Monocyte-Derived Macrophages

Timothy Smyth and Ilona Jaspers
Curriculum in Toxicology and Environmental Medicine, UNC Chapel Hill

Particulate matter (PM) exposure has been linked to reduced phagocytosis, reduced pathogen killing, and elevated pro-inflammatory cytokine expression in macrophages. Despite these previous findings, it is unclear whether PM has varying effects on different macrophage polarization states. We hypothesized that exposure to diesel exhaust particles (DEP), a major component of airborne PM, would reduce phagocytosis and increase pro-inflammatory cytokine secretion in a polarization state-dependent manner. Monocyte-derived macrophages were generated from the blood of healthy human donors (in compliance with the UNC IRB) and left unpolarized (M0) or polarized into pro-resolution M2 cells (20 ng/mL IL-4) for 24 hours. Following polarization, macrophages were exposed to M1-polarizing conditions (20 ng/mL LPS and IFN-γ) with or without diesel exhaust particles (DEP) for 24 hours. Following these exposures, phagocytosis of S. aureus bioparticles was assessed, or cell culture supernatants and RNA lysates were collected. Both M0 and M2 macrophages exposed to either M1-polarizing conditions or DEP demonstrated a significant reduction in phagocytosis while co-exposure induced a further reduction. Repolarized M2 and directly polarized M1 macrophages demonstrated similar gene expression profiles while DEP exposure induced comprehensive changes in gene expression patterns in both cell types, suggesting DEP exposure induces broad changes in gene expression patterns independent of initial polarization states. In contrast, macrophages repolarized from M2 to M1 states demonstrated a distinct intermediate M1/M2 cytokine secretion profile compared to directly polarized M1 cells, including significantly elevated secretion of several key pro-inflammatory cytokines compared to directly polarized M1 cells. While DEP exposure caused a shift in secretion patterns in both cell types, repolarized M2 cells maintained the mixed M1/M2 secretion profile. Together, this data suggest M2 macrophages are capable of repolarizing to a pro-inflammatory phenotype while retaining some of their original polarization programming. This mixed M1/M2 phenotype is characterized by elevated pro-inflammatory cytokine secretion with modified responses to DEP exposure, suggesting repolarized macrophages represent a unique population of cells in the context of inflammation and PM exposure.

Single Cell- and Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry Techniques for Studying the Uptake of Gadolinium Based Contrast Agents in *Chlamydomonas reinhardtii* Algae and *Arabidopsis Thaliana*

Presenter: Tyler Herek
C. Derrick Quarles Jr.,[1] Karolin Sommer,[2] Matthias Elinkmann,[2] Alexander Köhrer,[2] Sarah Reuter,[2] Tobias Becker,[2] Michael Sperling,[2] Uwe Karst[2]
[1]Elemental Scientific, Inc., Omaha, NE, USA; [2]University of Münster, Münster, Germany

Magnetic resonance imaging (MRI) plays a crucial part in medical diagnostics. Approximately 40% of all MRI examinations utilize Gd^{3+} as a paramagnetic ion due to its contrast-enhancing abilities. Therefore, gadolinium (Gd) is complexed within either a linear or macrocyclic ligand that is either ionic or non-ionic. It was determined in recent years that the linear gadolinium-based contrast agents (GBCAs) can lead to nephrogenic systemic fibrosis and also deposition of a small amount of Gd within the brain, which led to their ban in Europe in 2017.

For the most part GBCAs are not retained in the body and are excreted rapidly, which in turn releases them into the environment. Wastewater treatment plants only eliminate a small percentage of the GBCAs which means these complexes end up in our drinking water and surface waters, especially in the more densely populated regions. To further understand the potential uptake into our ecosystem, two different experiments were designed. First, the species-dependent uptake of GBCAs into Chlamydomonas reinhardtii algae which was investigated by single cell-inductively coupled plasma-mass spectrometry (sc-ICP-MS). This is a fairly new technique that introduces cells one at a time into the ICP-MS to get metal distributions, or in this case Gd, from cell populations. The second study investigated the uptake of free Gd^{3+}, linear GBCAs, and macrocyclic GBCAs in Arabidopsis Thaliana by laser ablation-ICP-MS (LA-ICP-MS). LA-ICP-MS is a technique that can be used for imaging and in this case is used to determine Gd distributions within the leaf that can help determine the amount of exposure and potential uptake paths.

Comparative health effects in male and female mice exposed to Libby Amphibole Asbestos

Adhikari U, Serve KM
Idaho State University

Asbestos is a fibrous silicate mineral that has been used in the construction and shipbuilding industry for over 200 years. Asbestos is classified as a carcinogen and can lead to serious diseases like lung cancer, malignant mesothelioma, and pleural fibrosis. Though all types of asbestos can cause respiratory and malignant diseases, only amphibole fibers are associated with autoimmunity. Several outcroppings of asbestos can be observed worldwide so it is important to understand the health effects due to environmental exposures. A previous study in mice was done mimicking environmental exposure (3 μ g/mouse) and examined long term (7 months) effects. Results showed increase in autoimmune markers and collagen deposition in lungs. We performed follow-up analyses of lungs collected from these mice and found colocalization of autoantibodies and citrullination. Citrullination is the post-translational modification of arginine to citrulline residues by peptidylarginine deiminase (PAD4) enzymes and is thought to be an essential contributor to autoimmune disease progression. Neutrophils and Macrophages produce PAD

enzymes and our recent study have shown the presence of extracellular PAD4 in cultured macrophages. To better understand the early responses that may lead to these long-term effects, our current study examines short-term (14 day) effects of low dose Libby Amphibole Asbestos (LAA) exposures. C57BL/6 male and female mice (n=6 each) were exposed by oropharyngeal aspiration to fiber suspensions at a dose of 3 μg/mice. Control mice were given an equivalent dose of saline. After 14 days of exposure, bronchoalveolar lavage (BAL) and pleural cavity wash were assessed for immune cell influx and cytokine concentrations. BAL was also examined for PAD4 and histologic analyses of lungs done to examine tissue citrullination. These data will help understand the short and long term effects of environmental exposure to asbestos and autoimmunity.

Changes in Protein Structure and Assembly with Fluoride Nanoparticles and Coexisting Ions

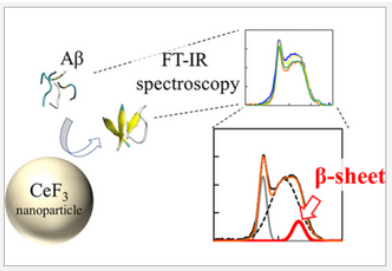
Masakazu Umezawa[1], Naoya Sakaguchi[1], Samal Kaumbekova[2], Mehdi Amouei Torkmahalleh[2], Dhawal Shah[2]

[1]Department of Materials Science and Technology, Faculty of Advanced Engineering, Tokyo University of Science, 6-3-1 Niijuku, Katsushika, Tokyo 125-8585, Japan

[2]Department of Chemical and Materials Engineering, School of Engineering and Digital Sciences, Nazarbayev University, Kabanbay Batyr 53, Nur-Sultan 010000, Kazakhstan

Protein function and activity is determined by both their assembly and secondary structure. Abnormalities related to either protein aggregation or secondary structure can lead to neurodegenerative diseases. This study investigated the potential effects of fluoride nanoparticles (NPs), materials used in in vivo imaging, on the assembly and structure of the amyloid β (Aβ) protein. Fourier transform infrared spectroscopy (FT-IR) was used to directly monitor the effect of fluoride (CeF3) NP surface on the amide bonds restricted by the peptides' secondary structure. Near the NP surface, Aβ16-20 peptides are more likely to form β-sheets. This comes as an effect of hydrophobic domain in the peptides. The parts of the peptide that repelled by the water solution stick to the NPs, and form aggregates more easily. Even without the NPs, the environment can affect the rate of secondary structure formation. The effect of NPs, resulting from a combination of electrostatic interaction and hydrogen bonding, was exaggerated upon adding CeF3 NPs. With a careful choice of ions and NPs, the β-sheet formation can be either suppressed or promoted. Especially, the formation of the β-sheet structure of Aβ peptides was promoted in the presence of NH_4^+, whereas it was suppressed in the presence of NO_3^- because of the electrostatic interaction between the lysine residue of the Aβ peptide and the ions. Our findings will contribute to comparative studies on the effect of different NPs with different physicochemical properties on the molecular state of proteins. Future works will develop processes that can be controlled and engineered to eradicate adverse effects of inorganic solid NPs.

Reference: N. Sakaguchi and M. Umezawa, et al., ACS Appl. Bio Mater., 5(6): 2843-2850



In vivo and *in vitro* toxicity of a stainless-steel aerosol generated during thermal spray coating

Vamsi Kodali[1,3], Aliakbar Afshari[1], Terence Meighan[1], Walter McKinney[1], Md Habibul Hasan Mazumder[3,4,5], Nairrita Majumder[3,4], Jared L. Cumpston[1], Howard D. Leonard[1], James B. Cumpston[1], Sherri Friend[1], Stephen S. Leonard[1], Aaron Erdely[1,3,4], Patti C. Zeidler-Erdely[1,3,4], Salik Hussain[3,4], Eun Gyung Lee[2], James M. Antonini[1,3]

[1] Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV; [2]Respiratory Health Division, National Institute for Occupational Safety and Health, Morgantown, WV; [3]Department of Physiology and Pharmacology, School of Medicine, West Virginia University, Morgantown, WV; [4]Center for Inhalation Toxicology (iTOX), School of Medicine, West Virginia University, Morgantown, WV

Thermal spray coating is an industrial process in which molten metal is sprayed onto a surface as a protective coating at high velocity. An automated electric arc wire thermal spray coating aerosol generator and inhalation exposure system was developed to simulate an occupational exposure and, using this system, male Sprague-Dawley rats were exposed to stainless steel PMET 720 thermal spray wire (~80% Fe, 15% Cr, 3% Mn, <1% Ni) aerosols at 25 mg/m3 x 4 hr/d x 9 d. Lung injury, inflammation, and cytokine level alterations were determined at 1, 7, 14 and 28 d after exposure. The aerosols generated were also collected and characterized. In a separate In vitro study, Macrophages were exposed to a wide dose range (0 - 200 μg PMET 720 particulate/ml) to determine cytotoxicity and to screen for known mechanisms of toxicity. Welding fumes were used as comparative particulate controls for the In vitro study. In vivo lung damage, inflammation and alteration in cytokine levels were observed 1 d post exposure and this response resolved by day 7. Alveolar macrophages retained the particulate even 28 days after exposure. In line with the pulmonary toxicity findings in vitro, cytotoxicity and membrane damage were observed only at the higher doses. Electron paramagnetic resonance showed that the particulate generated free radicals in an acellular environment. A dose-dependent increase in intracellular oxidative stress and NF-kB/AP-1 activity was observed in exposed macrophages. PMET 720 particles were internalized via clathrin- and caveolar-mediated endocytosis as well as actin-dependent pinocytosis/phagocytosis. PMET 720 thermal spray aerosols induced acute toxicity and the animals recovered from the acute pulmonary injury by 7 days post exposure.


See you next time!

ATTENDEE DIRECTORY

Ujjwal Adhikari
Idaho State University

John Adragna
NYU Langone

Devin Alewel
United States Environmental Protection Agency

Ankit Aryal
Louisiana State University

Roger Atanga
University of New Mexico School of Medicine

Reuben Attah
University of Utah

Justin Baca
University of New Mexico School of Medicine

Brenna Baird
University of New Mexico College of Pharmacy

Jessica Begay
University of New Mexico College of Pharmacy

Keith Bein
University of California, Davis

Barry Bleske
University of New Mexico College of Pharmacy

James Bonner
North Carolina State University

Larry Bowen
In-Tox Products, LLC

Gerrit Bredeck
IUF - Leibniz Research Institute for Environmental Medicine

Stephanie Brocke
University of North Carolina at Chapel Hill

Jared Brown
University of Colorado, Anschutz Medical Campus

Matthew Campen
University of New Mexico College of Pharmacy

Danielle Carlin
National Institute of Environmental Health Sciences

Jennifer Carranza
University of New Mexico School of Medicine

Flemming Cassee
RIVM: National Institute for Public Health and the Environment

Eliseo Castillo
University of New Mexico School of Medicine

Elena Cesa
University of Ferrara, NCS University - PHHI

Daniel Conklin
University of Louisville

Deborah Cory-Slechta
University of Rochester Medical Center

Josh DeAguero
Kidney Institute of New Mexico

Karol Dokladny
Kidney Institute of New Mexico

Seth Ebersviller
MedTec Biolab Inc.

Eliane El Hayek
University of New Mexico College of Pharmacy

Alison Elder
University of Rochester

Sherrie Elzey
TSI Inc.

Aaron Erdely
Centers for Disease Control / National Institute for Occupational Safety and Health

G.P. Escobar
Kidney Institute of New Mexico

Caleb Finch
University of Southern California

Kelly Fraser
Centers for Disease Control / National Institute for Occupational Safety and Health

Evan Gallagher
University of Washington

Marcus Garcia
University of New Mexico College of Pharmacy

Ian Gilmour
United States Environmental Protection Agency

Siem Goitom
University of New Mexico College of Pharmacy

Terry Gordon
New York University

Matthew Gorr
The Ohio State University

Kymberly Gowdy
The Ohio State University, DHLRI

Haiwei Gui
Florida International University

Jack Harkema
Michigan State University

Julie Harris
Kidney Institute of New Mexico

Hannah Hartzler-Lovins
The Ohio State University

Lauren Heine
Michigan State University

Guy Herbert
University of New Mexico College of Pharmacy

Tyler Herek
Elemental Scientific, Inc.

John Hoffman
Owens Corning

Kevin Hogeveen
ANSES

Russell Hunter
University of New Mexico College of Pharmacy

Salik Hussain
West Virginia University

Julie In
University of New Mexico School of Medicine

Thomas Jackson
United States Environmental Protection Agency

Annie Jarabek
United States Environmental Protection Agency

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University of North Carolina at Chapel Hill

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Columbia University

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University of Washington

Kamaljeet Kaur
University of Utah

Kerry Kelly
University of Utah

Rebekah Kendall
University of Montana

Maciej Klimiuk
Kidney Institute of New Mexico

Vamsi Kodali
Centers for Disease Control / National Institute for Occupational Safety and Health

Urmila Kodavanti
United States Environmental Protection Agency CPHEA PHITD

Tobias Krebs
VITROCELL Systems GmbH

Rahul Kumar
University of New Mexico School of Medicine

Gary Kurc
TSE Systems, Inc.

John Gregory Lamb
University of Utah

Mark Lawrence
SCIREQ

Pamela Lein
University of California, Davis

Selita Lucas
University of New Mexico College of Pharmacy

David Luglio
New York University Langone

Florian Maier
VITROCELL Systems GmbH

Roger McClellan
Independent Advisor, Toxicology and Risk Analysis

Shaun McCullough
United States Environmental Protection Agency

Kimberly McMullen
DSI, a division of Harvard Bioscience

Melanie Mendez
Lawrence Livermore National Laboratory

Christopher Migliaccio
University of Montana

Colette Miller
United States Environmental Protection Agency

Keith Murphy
DSI, a division of Harvard Bioscience

Alexander Nihart
University of New Mexico College of Pharmacy

Alexandra Noël
Louisiana State University

Tim Nurkiewicz
West Virginia University – Center for Inhalation Toxicology

Gunter Oberdorster
University of Rochester, NY, Medical School

Daniela Oettler
TSE Systems, Inc.

Mark Olfert
West Virginia University – Center for Inhalation Toxicology

Andrew Ottens
Virginia Commonwealth University

Juergen Pauluhn
Covestro AG, Germany

Arthur Penn
Louisiana State University

Laeticia Perez
Université Catholique de Louvain

Craig Poland
Centre for Inflammation Research University of Edinburgh

Christopher Reilly
University of Utah

Angela Reinert
University of Colorado, Anschutz Medical Campus

Cassandra Rice
University of Utah

Michaela Ritz
University of New Mexico College of Pharmacy

Katherine Roach
Centers for Disease Control / National Institute for Occupational Safety and Health

Jenny Roberts
Centers for Disease Control / National Institute for Occupational Safety and Health

Keegan Rogers
University of Colorado, Anschutz Medical Campus

Aaron Romero
University of New Mexico School of Medicine

Emily Ruggiano
University of Colorado, Anschutz Medical Campus

Katelyn Russell
The Ohio State University

Raul Alejandro Salazar
University of New Mexico College of Pharmacy

Jon Samet
University of Colorado

Katharina Schwarz
Fraunhofer Institute for Toxicology and Experimental Medicine

David Scieszka
University of New Mexico College of Pharmacy

Roel Schins
Leibniz Research Institute for Environmental Medicine

Kinta Serve
Idaho State University

Talia Seymour
Rutgers University

Jonathan Shannahan
Purdue University

Kristina Shkirkova
University of Southern California

Timothy Smyth
University of North Carolina at Chapel Hill

Marissa Sobolewski
University of Rochester Medical Center

Phoebe Stapleton
Rutgers University

Todd Stueckle
Centers for Disease Control / National Institute for Occupational Safety and Health

Matthew Sydor
Center for Environmental Health Sciences, University of Montana

Robert Taylor
University of New Mexico School of Medicine

Rachel Templeton
University of Pittsburgh

Masakazu Umezawa
Tokyo University of Science

Brent Wagner
Kidney Institute of New Mexico

Dave Wiederin
Elemental Scientific, Inc.

Loren Wold
The Ohio State University, DHLRI

Chieh-Ming Wu
The Ohio State University

Xiaozhong Yu
University of New Mexico

Jose Zavala
MedTec Biolab, Inc.

Ali Kermanizadeh
University of Derby

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