

and exposures to environmental stimuli are critical to disease prevention. Animal models, particularly inbred mice, provide important insight to understand human disease etiologies because genetic background and environmental exposures can be controlled. Tools including *in silico* haplotyping, collaborative cross and diversity outcross mouse panels, bioinformatic applications, and -omics technologies have enhanced our ability to identify disease genes and pathways to guide translational investigations that apply these discoveries to human populations. Combined genetic and genomic approaches have yielded important insight to mechanisms of susceptibility to many complex traits and diseases. We have integrated inbred mouse and cell-based models with haplotype association mapping (genetic), global gene expression analyses (genomic), and expression quantitative trait locus mapping (eQTL or genetical genomics) to identify candidate susceptibility genes and associated gene networks important in toxicant-induced lung injury. The overarching goal of these investigations is to determine whether human homologues of these susceptibility genes associate with disease risk in human populations. Efforts to identify and validate susceptibility genes in mouse models of environmental disease with a goal towards translational application have enabled identification of individuals who are susceptible to disease. For example, epidemiological and clinical investigations have associated functional polymorphisms in human *NRF2* (NF-E2 related factor 2) and *TNF* (tumor necrosis factor alpha) with susceptibility to acute lung injury and ozone-induced changes in lung function, respectively. Importantly, these discoveries may also lead to novel intervention or therapeutic strategies to prevent disease.

S 18 **Developmental Exposure to Bisphenol A and Lead: Effects on Metabolic Homeostasis and the Epigenome.**

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Environmental exposures during early development and other critical life stages may induce changes to the epigenome resulting in potentially deleterious phenotypic effects including metabolic disease, cancer, and neurological disorders. The field of epigenetics is experiencing a rapid advancement in technology, methodology, and data acquisition that now allows for the identification of the constellation of genomic loci with altered epigenetic status following dose-dependent exposures. Thus, epigenomic profiling facilitates the identification of biomarkers of exposure, enabling clinicians to identify at-risk individuals prior to disease onset. Utilizing a multi-pronged approach with an *in vivo* mouse model, human clinical samples, and an ongoing 15-year longitudinal epidemiological study, the overall goal of this presentation is to elucidate the impact of perinatal bisphenol A (BPA) and lead (Pb) exposure on metabolic homeostasis and DNA methylation, and the interplay between the two. Developmental exposure to environmentally relevant levels of BPA has been shown to affect both global and gene-specific DNA methylation patterns in rodents. We now draw upon data from multiple whole-epigenome platforms to show that multiple dose levels of BPA affect DNA methylation in mice and humans and that these epigenetic effects are non-monotonic in dose response. Preliminary studies also indicate that Pb exhibits epigenetic effects that may contribute to its known neurotoxic and obesogenic activities.

S 19 **Predictive Toxicology Paradigms for Understanding Carbon Nanotube Toxicity in the Lung.**

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Nanotechnology is rapidly developing, resulting in the production of a variety of engineered nanoparticles. Carbon nanotubes (CNTs) represent an important family of nanoparticles because they have many potential uses in engineering, electronics, and medicine due to their ease of functionalization, unusual strength, and electrical conductivity. However, these novel nanostructures also represent a potential human health risk, due to the possibility of inhalation exposure and evidence that the lung and cardiovascular systems are targets for hazardous effects. Inhalation studies in rodents show that CNTs deposit within the distal regions in the lungs and migrate to the pleura to cause inflammatory and/or fibrotic effects. Presentations in this session are aimed at elucidating the pulmonary and cardiovascular effects of CNTs, and how an increasing variety of functionalized CNTs can be evaluated via high-content screening. Because functionalized CNTs vary in toxicological activity, we will address high-content screening for the development of structure-activity relationships relevant to inhalation toxicity and safer design of nanoparticles. This will include exploration of factors that mediate toxic effects such as high aspect ratio, durability, and residual metal content and discuss how removing metal catalysts or changing surface properties alters the pattern and timing of toxicity. While the lung is a major target organ, another goal is to determine the potential for inhaled CNTs to have toxic effects that reach beyond the lung to influence the cardiovascular system. Finally, we will discuss how susceptibility factors,

both genetic and environmental, determine pulmonary and cardiovascular toxicity to CNTs. The outcome of this session is to gain a better understanding of the structure-activity relationships, target organs, and susceptibility factors that will aid the development of predictive toxicology paradigms for understanding CNT toxicity.

S 20 **Time Course of Pulmonary Responses to Inhaled Multiwalled Carbon Nanotubes.**

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In the present study, an aerosol of multi-walled carbon nanotubes (MWCNT) was produced with an acoustical generator, and airborne concentration and size distribution was determined. Mice were exposed by whole body inhalation to MWCNT (5 mg/m³, 5 hours/day, 12 days) and pulmonary responses were monitored at 1 day, 2, 4, 12, 24 and 48 weeks post-exposure. Pulmonary responses were investigated using whole lung lavage, histopathology, morphometry, and enhanced darkfield light microscopy studies. MWCNT lung burden was also measured to assess MWCNT clearance. Data indicate that the lung burdens of MWCNT in this study represent lung burdens relevant to estimated human occupational exposures and caused time-dependent pulmonary inflammation, damage and pulmonary fibrosis. Using enhanced darkfield microscopy, MWCNT fibers were found in lavage of the pleural space, parietal pleura, and respiratory muscles of the diaphragm and chest wall. The time course of pulmonary responses and their relationship to MWCNT lung burden and clearance will be discussed.

S 21 **Establishment of Carbon Nanotube Structure-Activity Relationships (SARs) That Can Be Used to Understand Pulmonary Toxicity and Safer Design.**

A. Nel. *UCLA, Los Angeles, CA.*

There is a fundamental gap in understanding how the physicochemical properties of carbon nanotubes (CNTs) contribute to hazard generation in the lung. Without this knowledge, it is difficult to evaluate CNT safety in a predictive manner. Our goal is to develop a predictive toxicological paradigm for CNT safety assessment in which we define the structure-activity relationships (SARs) leading to hazard generation at the nano/bio interface, including ways to design safer materials that do not induce chronic inflammation and fibrosis. To achieve this goal, we are developing a series of single-wall and multi-wall CNT test materials that can be screened by robust cellular assays to perform hazard ranking and SAR analysis. We are looking at the role of CNT dimensions (including length, diameter and aspect ratio), dispersability, catalytic surface chemistry, electronic properties and purity in initiating cooperative cellular interactions in macrophages and cells of the epithelial-mesenchymal trophic (EMT), which are involved in the pathogenesis of pulmonary inflammation and fibrosis. The above physicochemical characteristics impact the lysosomal stability in macrophages in a hierarchical fashion, leading to cathepsin B release and assembly of the subunits of the NALP3 inflammasome. This leads to IL-1 beta release, which primes the EMT unit and initiates a march of events leading to TGF-beta and PDGF production and subsequent induction of chronic inflammation and fibrosis in the lung. Utilizing myeloid and epithelial cell lines, it is possible to study the induction of these biomarkers in relation to the property variations of the CNT materials, predicting the SARs that are associated with pulmonary inflammation and fibrosis. Moreover, we have also implemented surface coating and functionalization approaches that can change the hazardous characteristics, leading to the design of safer CNTs. The overall utility of this research exploration is to establish a predictive and quantitative toxicological paradigm for the safety assessment of CNTs and their safe implementation in the marketplace.

S 22 **Surface and Chemical Modification of Single-Walled Carbon Nanotubes Does Not Necessarily Create a Safer Nanomaterial.**

K. E. Pinkerton. *University of California Davis, Davis, CA.*

We hypothesized iron (Fe) content and morphology of inhaled single-walled carbon nanotubes (SWCNTs) would influence the extent of cellular injury and alters homeostasis in the lung. Rats (SD) were exposed (1 mg/m³) to either aerosolized

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