PS 237 Diacetyl-Induced Respiratory and Olfactory Toxicity in Mice: Influence of Ubiquitination, Gender, and Dicarbonyl/L-Xylulose Reductase Gene Knockout.

A. F. Hubbs[1], K. L. Fluharty[1], M. P. Goravanahally[2, 1], R. J. Edwards[2, 1], M. L. Kashon[1], L. Sargent[1], R. R. Mercer[1], M. C. Jackson[1], A. M. Cumpston[1], W. T. Goldsmith[1], J. S. Fedan[1], R. D. Dey[2], L. A. Battelli[1], T. Munro[1], W. B. Moyers[1], P. A. Willard[1], K. McKinstry[1], S. Friend[1] and K. Sriram[1]. *[1]HELD, NIOSH, Morgantown, WV; [2]West Virginia University, Morgantown, WV.*

The α-dicarbonyl butter flavoring, diacetyl (2,3-butanedione), is associated with flavorings-related constrictive bronchiolitis in workers who make or use flavorings. Diacetyl causes protein damage in a process believed to be dependent upon the α-dicarbonyl structure. A protective response to damaged protein is ubiquitination with subsequent proteasomal processing. Diacetyl is also metabolized to the less reactive α-hydroxyketone, acetoin, by dicarbonyl/L-xylulose reductase (Dcxr). We examined the role of Dcxr and gender on acute toxicity of inhaled diacetyl by exposing Dcxr knockout and wildtype mice of both sexes to diacetyl at target concentrations of 0, 100, 200 or 300 ppm for 6 hr. At 1 day post-exposure, endpoints were semi-quantitative histopathology and morphometric measurement of ubiquitin immunofluorescence in nose and lung sections. Ubiquitin was principally localized to nasal and intrapulmonary airways, increased in large bronchioles at concentrations ≥ 100 ppm, and in the nose at 300 ppm. Diacetyl-induced ubiquitin in the nose and lung was modified by both gender and Dcxr. In lung histopathology, diacetyl caused vacuolation of airway epithelium of large bronchioles at concentrations ≥ 100 ppm. In olfactory bulb (OB) of male mice inhaling 300 ppm diacetyl, mRNA expression of inflammatory mediators and olfactory marker protein (Omp), a marker of olfactory neuron axons, were assayed by real-time PCR. Diacetyl elevated Il6, Cxcl2, and Tnfa and decreased Omp in OB. The data suggest that ubiquitin expression is a sensitive biomarker of diacetyl-induced protein damage in airway epithelium. Further, diacetyl causes neuroinflammation and potential loss of axons of olfactory neurons in OB, suggestive of neurotoxicity.

PS 238 Subchronic Exposure to Ambient Particulate Matter Induces Oxidative Stress Responses in Brain Tissue of ApoE-/- Mice.

L. B. Mendez[1], A. J. Keebaugh[1], L. Chen[2], M. Lippmann[2] and M. T. Kleinman[1]. *[1]University of California Irvine Irvine, CA; [2]New York University, New York, NY.*

Exposure to particulate matter (PM), present in urban environments, has been shown to induce pro-inflammatory and oxidative stress responses in the central nervous system (CNS) of apopoliprotein E knockout (ApoE-/-) and Balb/c mice. In this study oxidative stress responses in different subcellular fractions of the ApoE-/- mouse brains were evaluated after a subchronic exposure to fine (≤2.5 μm) concentrated ambient particles (CAPs). Apo E-/- mice were exposed to either CAPs or particle-free air for 5 hours a day, 5 days per week, for a period of 6 months. The whole-body inhalation exposures were conducted in two urban cities (Seattle, WA and Detroit, MI) with distinct sources and chemical composition of PM. Brain tissue was collected after the exposures were completed and analyzed for biomarkers of oxidative stress. The antioxidant glutathione was reduced in the brains of mice exposed to CAPs in Michigan but not in Washington. In contrast the lipid peroxidation product 4-hydroxyalkenal (HNE) was significantly increased in the membrane fraction of brain tissue of mice exposed to CAPs in Washington but not in Michigan. No significant differences were observed in protein carbonyl levels, a biomarker of protein oxidation, although the levels were slightly higher in the cytoplasmic fraction of brain tissue from animals exposed to CAPs when compared to controls regardless of exposure site. The results suggest that PM from different sources can modulate oxidative stress responses in a distinct fashion and that different subcellular fractions in the brain can be more susceptible to the effects of PM.

PS 239 Co-Exposure to Ultrafine Particulate Matter and Ozone Causes Electrocardiogram Changes Indicative of Increased Arrhythmia Risk in Mice.

N. Kurhanewicz[1], R. McIntosh-Kastrinsky[1], L. Walsh[2], A. K. Farraj[2] and M. S. Hazari[2]. *[1]University of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Environmental Public Health Division, US EPA, Research Triangle Park, NC.*

Numerous studies have shown a relationship between acute air pollution exposure and increased risk for cardiovascular morbidity and mortality. Due to the inherent complexity of air pollution, recent studies have focused on co-exposures to better understand potential interactions. This study was designed to evaluate the cardiac effects of concentrated ambient fine (PM2.5) and ultrafine (UFP) particles with and without ozone (O3) co-exposure. We hypothesized that ozone co-exposure would enhance the acute effects of particles, particularly UFP. Conscious unrestrained C57BL/6 mice implanted with radiotelemeters were exposed by whole-body inhalation to either 250 ug/m3 PM2.5 or 100 ug/m3 UFP with or without 0.3 ppm O3 (4hrs); separate groups were exposed to either filtered air or O3 only. Heart rate (HR) and electrocardiogram (ECG) were recorded continuously before, during and after exposure. Control animals experienced a decrease in HR during exposure. Neither PM2.5 nor UFP alone caused any HR change; however with O3 co-exposure, HR remained transiently elevated above control levels. Exposure to UFP+O3 caused decreased PR-interval, a transient increase in QRS, and increased QTc. PM2.5 alone caused QRS to decrease and O3 alone caused a decrease in QRS interval and QTc. There were no other significant differences in the ECG parameters measured of any groups. Lastly, only animals exposed to UFP+O3 had an increase in the number of non-conductive P-waves; there were no differences in other arrhythmia counts. These data suggest that O3 co-exposure might worsen the stress response to PM, especially UFP, and cause repolarization heterogeneity in the heart, which increases the risk for arrhythmogenesis. As such, this indicates that the cardiovascular effects of particle and gas co-exposures are not easily characterized, potentially increasing the complexity of risk assessment. (This abstract does not reflect EPA policy)

PS 240 An Air-Liquid Interface *In Vitro* Approach for Studying Toxic Effects of Exhaust Emissions Using a Heavy Duty Truck.

I. M. Kooter[1], M. Alblas[1], J. van Triel[2], A. Jedynska[1], M. Steenhof[1], M. Houtzager[1] and M. van Ras[1]. *[1]EELS, TNO, Utrecht, Netherlands; [2]TAP, TNO Triskelion, Zeist, Netherlands.* Sponsor: R. Woutersen.

Classically in vitro diesel exhaust (DE) studies have been performed with submerged cell cultures which are exposed to collected DE particles. Major drawback of this exposure method is that cells are not exposed to the whole complex and dynamic mixture of compounds DE is composed of. In recent years, air-liquid interface exposures have become more widely used, enabling in vitro exposures to mixtures of gases and particles. The main objective of this study was to investigate the feasibility of exposing human lung epithelial cells at the air-liquid interface to complete DE generated by a heavy-duty truck in the state-of-the-art TNO power train facilities.
Human epithelial lung cells (A549) were directly exposed at the air liquid interface to DE generated by a heavy-duty Euro III truck (turbo diesel model 2002). The truck was tested at a steady-state cycle at a speed of ~70 kmh-1 to simulate free-flowing traffic at a motorway on a transient engine dynamometer. Cells were exposed to DE for 1.5 hours. After a 24 hours post-incubation period, cells were analysed for markers of oxidative stress (glutathione levels, GSH; heme oxygenase 1 protein levels, HO-1), cytotoxicity (lactate dehydrogenase release, LDH; Alamar Blue assay) and inflammation (interleukine-8 protein levels, IL-8).
DE exposure resulted in a decreased cell viability (significantly decreased Alamar Blue levels in the incubation medium and slightly increased LDH levels), and an increased oxidative stress response (significantly increased HO-1 levels and reduced GSH/GSSH ratio). However, the pro-inflammatory response seemed to decrease (non-significant decrease in IL-8).
The results presented here demonstrate that our in vitro exposure approach is indeed well suited for testing complex particulate and gaseous pollutant mixtures from diesel trucks. Our results confirm previous in vitro studies showing cytotoxicity and oxidative stress responses due to DE exposure.

PS 241 Comparative Toxicity of Soy Biodiesel and Diesel Emissions in Healthy and Allergic Mice.

S. H. Gavett[1], M. A. Williams[1], J. M. Cyphert[2, 1], E. H. Boykin[1], M. E. Daniels[1], L. B. Copeland[1], D. L. Andrews[3], J. H. Richards[1] and I. Gilmour[1]. *[1]EPHD, NHEERL, US EPA, Research Triangle Park, NC; [2]Curriculum in Toxicology, UNC School of Medicine, Chapel Hill, NC; [3]Research Cores Unit, Proteomic Research Core, US EPA, Research Triangle Park, NC.*

Toxicity from combustion of 100% soy-based biodiesel (B100) was compared to that of petrodiesel (B0) or a 20% biodiesel / 80% petrodiesel mix (B20) in healthy and house dust mite (HDM)-allergic Balb/cJ mice. Exhaust from combustion of B0, B20, or B100 was diluted to target concentrations of 50, 150, or 500 μg/m3 as determined by real-time Tapered Element Oscillating Microbalance. Studies in healthy mice showed greater levels of MIP-2 and neutrophils in bronchoalveolar lavage (BAL) fluid 2 hr after a single 4 hr exposure to B0 compared with exposure to biodiesel emissions (air control neutrophils = 1x, B0 = 11.9x, B20 = 4.4x, B100 = 2.1x). However these differences were attenuated 24 hr after exposure and no consistent differences were observed 2 or 24 hr after 5 d (4 hr/d) or 4 wk (5 d/wk) exposures. Mice sensitized and challenged intranasally with HDM and exposed to B0, B20, or B100 for 4 wk (5 d/wk) had no emissions-related differences in airway

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