**OZONE PRIMING AFFECTS THE LUNG RESPONSE TO ULTRAFINE PARTICLES AND LPS.** CJ Johnston, JN Finkelstein, ACP Elder, N Corson, P Mercer, R Gelein, G Oberdoerster. The University of Rochester, Departments of Environmental Medicine and Pediatrics (Neonatology), Rochester, NY.

Epidemiologic evidence links episodes of elevated levels of airborne particulate matter (PM) with increases in morbidity and mortality. At particular risk are individuals with pre-existing lung injury or disease. This may indicate that the lung is primed by earlier events so that subsequent exposure to PM triggers events such as cytokine release. Eight week old C57BL/6 mice were exposed to 0.5 ppm $O_3$ for 24 hours, and additionally during the final 6 hours to ultrafine carbonaceous particles (UP) at ~100 $\mu g/m^3$. Other groups of mice were exposed to $O_3$ or UP alone or low dose inhaled LPS or to various combinations of these compounds. Two hours post $O_3$ exposure, messages encoding Eotaxin, MIP-1α, MCP-1, IL-6, TNFα and CCR-1 were elevated. Two hours post UP exposure, messages encoding MIP-1α, MCP-1 and CCR-1 were elevated. Two hours post LPS exposure, messages encoding Eotaxin, MIP-1α, MIP-1β, MIP-2, MCP-1, TNFα, IL-6 and CCR1 were elevated. Two hours post exposure to 24 hours of 0.5 ppm $O_3$ followed by LPS inhalation, induced an additive response for messages encoding IL-6, MCP-1, CCR-1 and TNFα. These results suggest that pre-exposure to ozone, which primarily attacks the epithelium can cause sensitization to a secondary stimulus.

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CELL PROLIFERATION RESPONSES FOLLOWING SINGLE AND REPEATED EXPOSURE TO PARTICULATE MATTER AND OZONE IN NEONATES, YOUNG ADULTS AND SENESCENT RATS. D.M. Hyde, L.F. Putney, M.Y. Stovall, L.A. Miller, B.K. Tarkington and K.E. Pinkerton. Center for Comparative Respiratory Biology and Medicine, University of California, Davis, CA 95616

Epithelial injury induced by ozone ($O_3$) inhalation is site specific in the airways. Particulate matter (PM) about 1 μm diameter deposits in similar airways targeted by $O_3$. To define patterns of injury and repair in airways to PM in three different ages of F344 rats (neonates, young adults and senescent), we exposed rats in four groups to 1) filtered air, 2) PM ($NH_4NO_3$+carbon, MMAD 1.0μm; low = 77+59 $\mu g/m^3$, medium = 151+125 $\mu g/m^3$ or high = 264+201 $\mu g/m^3$, respectively), 3) $O_3$ (0.2 ppm) or 4) PM with $O_3$ for 6 h or 6 h per day for 3 days. An osmotic minipump loaded with BrdU was placed subcutaneously in rats 12 h prior to exposure. Right cranial lung lobes from rats were microdissected along the primary airway long axis. We used an optical disector to count the number of BrdU-positive cells per volume of epithelium or interstitium of bronchial bifurcations, TBs and alveolar ducts. A significant increase in BrdU-labeled TB epithelium in young adults at the intermediate PM dose alone or with ozone was observed after a single exposure indicating a potential compensatory effect at high PM concentrations. In contrast, repeated exposure showed significant increases in BrdU-labeled TB interstitial cells only in neonates at the high PM concentration alone or with ozone. Our data suggests that the effects of PM are independent of the influence of $O_3$ and age-specific. Further, the ability to compensate for PM doses at environmentally relevant concentrations in neonates may be compromised.

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INHALATION OF ULTRAFINE PARTICLES HAS NO EFFECT ON IMMUNE RESPONSE AND AIRWAY REACTIVITY IN A BEAGLE DOG MODEL OF ALLERGIC ASTHMA. K. Rudolph, L.E. Bowen, B.A. Muggenburg, M. Nysus, and D.E. Bice, Lovelace Respiratory Research Institute, Respiratory Immunology Program, Albuquerque, NM, USA.

Our data indicate that the dog is an excellent model to study the role of pulmonary immunity in asthma. Using this model, we evaluated the effects of particle inhalation on immunity and airway reactivity to ragweed. Six allergic and 6 non-allergic dogs were exposed to ultrafine carbon black particles (232.3±2.5 $\mu g/m^3$, 35.2±0.3 nm) for one hour, followed by a challenge of water as a control. Airway resistance was measured during particle exposure and after water challenge. Immunity before and after particle exposure was assessed by measuring total IgE, and ragweed-specific IgE and IgG in serum and bronchoalveolar lavage fluid (BALF), and cell differentiation in BALF. Each dog was exposed a second time to carbon particles (251.4±5.3 $\mu g/m^3$, 34.9±0.5 nm) followed by a challenge of ragweed and the same variables were measured. Pulmonary resistance did not change during particle exposure in any of the dogs, and ragweed-induced airway reactivity was not altered by particle exposure. Total and ragweed-specific serum IgE and total IgE in BALF were higher in allergic dogs at all time points. Particle exposure did not affect antibody levels in serum or BALF in allergic dogs. Non-allergic dogs developed specific IgG in response to multiple inhalation exposures to ragweed, but this was not related to the utrafine particle exposure. Particle exposure did not alter the numbers of eosinophils. Neutrophils in BALF were elevated in all groups one day after particle exposure. In conclusion, inhalation of ultrafine particles did not affect the immune response or airway reactivity in control or allergic dogs, although a low level of inflammation was induced in both allergic and non-allergic dogs. Research conducted by the National Environmental Respiratory Center (NERC) with support from multiple government and industry sponsors, including the U.S. EPA. This abstract is not intended to represent the views or policies of any NERC sponsor.

PULMONARY INFLAMMATION IN RATS EXPOSED TO CONCENTRATED AMBIENT PARTICLES (CAPs) IS ASSOCIATED WITH COMBUSTION COMPONENTS. Saldiva, P.H.N., Clarke, R.W., Stearns, R.C., Lawrence, J., Koutrakis, P., Godleski, J.J. Harvard School of Public Health, Boston, MA, USA

The objectives of this study were: 1) to verify that exposure to CAPs causes pulmonary inflammation; 2) to determine the anatomical location of the inflammatory reaction within the lungs; and 3) to assess the role of particle composition on the magnitude of the inflammatory reaction. Rats were exposed either to filtered air (S, n=11) or CAPs (C, n=13) using the Harvard ambient particle concentrator during 3 consecutive days (5h/day) in 5 distinct periods of exposure (mean $PM_{2.5}$ = 285 $\mu g/m^3$, range 74 to 733). $PM_{2.5}$ samples were collected for elemental analysis (XRF), elemental carbon, and sulfate concentrations. 24 hours after the last exposure, the lungs were fixed at constant pressure (25cm$H_2O$) with 2.5% glutaradehyde. Random transverse sections were embedded in paraffin and 5-μm thick H&E slides were coded for blinded analysis. The numerical density of neutrophils (Nneu) within the alveolar septa was determined in 10 random microscopical fields – 5 in the centri-acinar region (CA) and 5 in the peripheral portion of the pulmonary acinus (PER) - for each lobe. The amount of solid pulmonary parenchyma (SP) was determined in the same fields by point-counting, and Nneu was corrected for SP. Statistical analysis used generalized linear models. CAPs induced a significant increase (p=0.024) in Nneu, mainly in CA (C=0.258±0.086 C vs S=0.190±-0.102) and to a lesser extent, in PER (C=0.196±0.069 vs S=0.159±0.096). In CAPs exposed rats, Nneu was significantly correlated with the concentration of Br (p=0.013), Pb (p=0.022); Zn (0.011), Mn (p=0.023) and elemental carbon (p=0.022). Our results indicate that CAPs exposure elicits acute inflammation mostly in the centri-acinar region; and intensity of the inflammation is influenced by elements indicative of combustion processes.

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EFFECTS OF DIESEL EXHAUST PARTICLES (DEP) EXPOSURE ON PULMONARY CYTOCHROME P-450 (P-450) METABOLISM. J. Y. C. Ma[1], E. Kane[2], M. W. Barger[1], and V. Castranova[1]. [1]NIOSH, Morgantown, WV, USA; [2]Shepherd College, Shepherdstown, WV, USA.

Polycyclic aromatic hydrocarbons (PAHs) associated with DEP require bioactivation by P-450 to exert their toxic/carcinogenic effects. The objective of the present study was to determine the effects of exposure of rats to DEP on pulmonary P-450 metabolism. Rats were dosed with a single intratracheal instillation of either 5 or 35 mg/kg of DEP or carbon black (CB). CB was used as a particle control without adsorbed organics. Rats were sacrificed 3 days after exposure, and pulmonary microsomes were isolated by differential centrifugation of lung homogenates. The protein level and activity of P-450 isozymes, CYP1A1 and CYP2B1, were monitored to assess the effects of DEP exposure on the P-450 enzyme system. CYP2B1 and CYP1A1 activities were measured by the O-dealkylation of 7-pentoxyresorufin and 7-ethoxyresorufin, respectively. The protein levels were determined by Western blot analysis. At both exposure doses, DEP or CB significantly reduced CYP2B1 activity, while only high dose exposure lowered CYP2B1 protein level. At 5mg/kg of DEP or CB, CYP1A1 activity was significantly lowered without affecting the protein level. At the 35mg/kg exposure level, DEP significantly increased CYP1A1 activity and protein level, whereas CB reduced CP1A1 activity and had little effect on the protein level. These data suggest that the effects of DEP on pulmonary P-450 are two fold: an induction of CYP1A1 by PAHs, the organic components of DEP, and a reduction of CYP2B1 activity due for the most part to the carbonaceous core of the particles. Both effects may play important roles in DEP induced toxicity.

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**OXIDATIVE DNA DAMAGE IN ALVEOLAR EPITHELIAL CELLS BY AMBIENT PARTICULATE MATTER.**

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Besides acute respiratory and cardiovascular effects, ambient particulate matter (PM) has also been associated with lung cancer mortality. Genotoxicity research on PM has merely focused on the effects of particle extracts (e.g. mutagenicity of polycyclic aromatic hydrocarbons), but this approach fails to evaluate PM as an entirety. We have investigated oxidative DNA damage by coarse ($PM_{10-2.5\mu m}$) and fine ($PM_{<2.5\mu m}$) PM. Electron spin resonance measurements and acellular experiments with calf thymus DNA demonstrated respectively, that both coarse and fine PM as well as their water-soluble fractions elicit transition-metal dependent hydroxyl radical formation and induction of 8-hydroxy-deoxyguanosine (8-oxodG). PM also caused a dose-dependent oxidative DNA damage in rat and human alveolar epithelial cell lines (RLE, A549), as shown by immunocytochemistry of 8-oxodG and by the comet assay. Remarkably, damage by the PM suspensions appeared to be significantly stronger than that of their corresponding extracts. DNA damage was also determined in relation to particle uptake (electron microscopy), transition metal leaching (IC-PMS), and in the presence of desferoxamine, N-acetyl-cysteine, and the lipid peroxidation inhibitor U-101033E. Our data demonstrate that the water soluble fraction of PM elicits oxidative DNA damage via transition metal dependent OH-formation, and indicate the contribution of a direct oxidative 'particle' effect to the genotoxic hazard of ambient particulate matter.

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