2h ($p<0.001$) and 24h ($p<0.05$) post-exposure, respectively. Increased chemiluminescence in lung tissue protein extracts correlated with elevated levels of luciferase protein. Bioluminescent staining of lung and heart frozen tissue sections demonstrated localized areas of oxidative stress. RNA was recovered from these regions of interest following laser capture microdissection. Gene expression profiling by microarray analysis of recovered RNA samples is currently ongoing. These results demonstrate the ability of air pollution particles derived from oil combustion to induce local and systemic oxidative stress which in turn may result in acute organ injury as well as potentially contribute to disease processes that have oxidative stress as a common pathological etiology. Funding: EPA/NCSU Training Agreement CT826512 to E. Roberts. (This abstract does not reflect EPA policy)

1445 CONCENTRATED AMBIENT AIR POLLUTION CREATES OXIDATIVE STRESS IN CNS MICROGLIA.

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Particulate matter (PM) is known to affect extrapulmonary targets. Nanometer size PM particles carry free radical activity on their surface and damage target tissues through oxidative stress (OS). Recently, we've reported a 29% reduction of CNS neurons (i.e., n. compacta of the substantia nigra) in a transgenically modified mouse strain (Apo $E^{-/-}$) exposed for 5 mo to concentrated northeastern regional background ambient particles (CAPs) (Veronesi et al., Inhalalation Toxicology in press). Since these neurons are highly sensitive to OS mediated damage, the response of microglia (the brain macrophage) to the same CAPs was examined. Immortalized, mouse microglia (BV2) were exposed to high and low potency CAPS (as determined by NF-kB activity) and endpoints, indicative of oxidative stress mediated inflammation, were collected. ATP levels, assayed with Luciferase-based chemiluminescence, were significantly reduced after 5 min exposure to >250 μg/ml mitochondrial depolarization after 30 min exposure. Significant increases in TNF α, IL6 and IL1 β were shown after 6 hr exposure. Glutathione and non-protein sulfhydryl levels, markers of OS were also significantly increased at 25-100 μg/ml concentrations. Cells, exposed for 4 hrs to 75 μg/ml of the high or low potency CAPs, were analyzed for genomic expressions, using an Affymetrix microarray. Bioinformatics indicated highly significant changes in genes associated with cell cycling, apoptosis, adhesion molecules, NF-kB, Toll receptors, Superoxide dismutase, proinflammatory cytokines, oncogenes, TNF receptors and others. Taken together, these data indicate that CNS microglia respond to concentrated ambient PM with changes suggestive of OS mediated inflammation. (This abstract has been reviewed by the USEPA, NHEERL and does not necessarily reflect its policy).

1446 EFFECTS OF PARTICULATE MATTER ON THE PULMONARY AND VASCULAR SYSTEM: TIME COURSE IN SPONTANEOUSLY HYPERTENSIVE RATS.

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Within the scope of two multi center projects, "Health effects of particles from motor engine exhaust and ambient pollution" (HEPMEAP) and "Chemical and biological characterization of ambient air coarse, fine, and ultrafine particles for human health risk assessment in Europe" (PAMCHAR), the present study aimed to optimize the design for PM toxicity screening studies in terms of dose and time between a single intratracheal exposure and the determination of the biological responses in compromised rats. PM was sampled at a road tunnel (RTD) using a high volume cascade impactor. Spontaneously hypertensive rats were exposed to EHC-93 or different doses of RTD. Autopsy was performed at 4, 24 or 48 hrs post-exposure. The neutrophil numbers in bronchioalveolar lavage fluid increased tremendously after exposure to relatively high RTD concentrations (10-15 fold) or EHC-93 (25 fold). Furthermore, PM exposure affected blood coagulation since plasma fibrinogen levels were increased significantly (1.2 fold). Pulmonary inflammation, oxidative stress, and changes in blood coagulation factors as well as circulating blood cell populations were observed within the range of 3 to 10 mg PM/kg body weight without the presence of significant pulmonary injury. At a lower dose only some inflammatory effects can be detected, which will probably be too small to discriminate between PM samples, and potential confounding effects were observed with the highest dose. In addition to dose, 24 hrs post-exposure seemed to be the appropriate time to assess relative toxic potency of PM since the majority of the health effects were observed one day after PM exposure compared to the other times examined. Aforementioned considerations provide a good foundation for the design of PM toxicity screening studies.

1447 ENDOTHELIAL INJURY IN PARTICULATE MATTER (PM)-INDUCED CARDIOVASCULAR INJURY: KINETIC ANALYSIS OF GENE EXPRESSION PROFILES.

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Numerous epidemiological studies established positive associations between ambient fine PM and cardiovascular morbidity and mortality. The biological basis for these adverse health effects is yet to be elucidated. Cardiovascular toxicity of fine PM and its toxic constituents may be direct or indirect. Vascular endothelial cells have been implicated in both direct and indirect cardiovascular effects of PM. To understand the role of endothelial injury and dysfunction in PM cardiovascular toxicity, we have initiated *in vitro* studies using primary human vascular endothelial cells. Acute exposure (25min) to a very low concentration (1mg/ml) of a model emission source PM, residual oil fly ash (ROFA) indicated differential gene expression profile for genes representing various functional groups such as cytokines, growth factors, vascular tone regulators, adhesion molecules, transporter proteins and voltage gated signaling mediators. Molecular observations observed at this early time point predicted alterations in plasma membrane structural and functional properties. Cytological observations at 8h post exposure indicated a role for these genes in the endothelial injury. To further understand the role of continuous exposure of endothelial cells to the same concentration of ROFA, we carried out exposure time kinetic studies at 25 min, 1, 3, 8, 12 and 24h. Gene expression profiles analyzed for ~20, 000 genes using human Affymetrix gene chips (H133A) at these time points clearly show time-dependent differential gene expression profiles consistent with progressive endothelial injury and dysfunction. The results of this study may provide insight to understand the role of the endothelium and the molecular basis for PM cardiovascular toxicity. It also may provide a biological basis for the discrepancies observed on the onset of acute myocardial events in cardiac patients associated with that ambient PM exposures. (This abstract does not reflect USEPA policy).

1448 AIRBORNE PARTICULATE MATTER UPREGULATES INFLAMMATORY MARKERS IN THE MOUSE BRAIN: IMPLICATIONS FOR NEURODEGENERATION.

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Neurodegenerative diseases are multifactorial. Senescence is a common predisposing factor. However, genetic mutations and environmental factors have also been linked to the onset and progression of these disorders. While the pathological hallmarks of different age-related neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis are unique, certain underlying processes appear to be generally enhanced. These include oxidative and inflammatory events. Concentrated particulate matter present in polluted air enhances proinflammatory markers in the brain. The activated levels of the immune related transcription factor NF-κB were increased. Furthermore, the concentration of the cytokines IL-1α and TNF-α were elevated. These effects were not due to the presence of endotoxin in the particulate matter. It is possible that this environmental exposure can trigger an innate-immune response in the CNS and by doing so aggravate already existing age-related adverse events. Transgenic models of neurodegenerative disorders can be an essential tool in determining whether environmental exposures do indeed contribute to and exacerbate pathological lesions associated with age-related disorders.

1449 SOLUBLE NICKEL ASSOCIATED WITH RESIDUAL OIL FLY ASH INCREASES SUSCEPTIBILITY TO PULMONARY INFECTION IN RATS.

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Soluble metals of residual oil fly ash (ROFA), an air pollutant from the combustion of fossil fuel, may be associated with lung infection and morbidity in susceptible populations. The present objective was to determine which soluble metal in ROFA was associated with decreased clearance of bacteria from the lungs of rats. At day 0, rats were intratracheally instilled (IT) with soluble $NiCl_2$ (55.7μg), $FeSO_4$ (37.2μg), $AlSO_4$ (46.6μg), $ZnCl_2$ (8.69μg), or a mixture of the metals in quantities present in a 2.0 mg dose of ROFA as determined by elemental analysis. On day 3, rats were infected with an IT dose of $5x10^4$ *Listeria monocytogenes* and euthanized on days 6, 8 and 10. The left lungs were homogenized to assess bacterial load, and bronchoalveolar lavage (BAL) was performed on the right lungs to measure lung injury and inflammation. Cells from lymph nodes were analyzed to determine phenotype. On day 6, rats exposed to Ni or the metal mixture had an increased bacterial lung burden as compared to all groups, and rats exposed to Zn had a higher bacterial load when compared to rats exposed to Al, Fe, or saline control. Lactate

dehydrogenase and albumin levels in BAL fluid were 3 times greater in the Ni and mixed metal groups on day 6 as compared to controls. Treatment with Ni and the metal mixture resulted in a 3 and 2 fold increase, respectively, in the number of cells in the BAL at all time points. The number of T cells in the BAL of the Ni and the mixed metal groups was decreased on day 6 and the number of natural killer cells was decreased in the Ni group at all time points as compared to controls. The ratio of $CD4^+$ to $CD8^+$ T cells in the BAL and in the lymph nodes was higher in the Ni and mixed metal groups on day 6. In summary, rats treated with soluble Ni, alone or in a mixture with soluble Fe, Zn, and Al, showed increased lung injury, decreased bacterial clearance, and altered lymphocyte profiles at early time points post-infection. Thus, soluble Ni may play an important role in the increase in susceptibility to infection after ROFA exposure.

1450 MEMBRANE PERMEABILITY AS A DETERMINANT OF ZN^{2+}-INDUCED SIGNALING IN HUMAN AIRWAY EPITHELIAL CELLS.

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Zinc (Zn^{2+}) is an essential micronutrient but also a common metallic constituent of ambient air particulate matter(PM) that may play a role in PM-induced adverse health effects. *In vivo* and *in vitro* studies have shown that Zn^{2+} exposure induces inflammatory responses in the airways. We have previously shown that exposure to Zn^{2+} results in activation of multiple intracellular signaling intermediates, including the epidermal growth factor receptor(EGFR), in a variety of cell types. Based on this variability, we hypothesized that cellular permeability is a factor limiting the magnitude of signal transduction activation induced by Zn^{2+}. To test this hypothesis, we used the Zn^{2+} ionophore pyrithione to permeabilize the human bronchial epithelial cell line BEAS and primary human airway epithelial cell cultures(HAEC) to Zn^{2+} and measured levels of EGFR phosphorylation. Treatment of BEAS cells with sub- to low-micromolar concentrations of pyrithione in the presence of 250 uM extracellular Zn^{2+} resulted in dose-dependent increases in Zn^{2+}-specific fluorescence as determined by fluorometry using FURA-2. Lysophosphatidylcholine, a physiologically relevant permeabilizing agent, also increased Zn^{2+} permeability in BEAS cells. Co-administration of 4 uM pyrithione markedly potentiated Zn^{2+}-induced EFGR phosphorylation at the auto- and trans-phosphorylation sites Y1068 and Y845, respectively, in HAEC. These findings show that Zn^{2+} acts through an intracellular target to effect EGFR phosphorylation. Further, cellular permeability is a critical determinant of cellular responsiveness to signal initiation by exposure to Zn^{2+}. Pathophysiological alterations in cellular homeostasis that may result in increased permeability to Zn^{2+} influx and sensitize HAEC to the effects of inhaled Zn compounds are presently under investigation. Additionally, the possibility that xenobiotics with pyrithione-like properties exist in PM is also being studied. THIS ABSTRACT OF A PROPOSED PRESENTATION DOES NOT NECESSARILY REFLECT EPA POLICY.

1451 ZINC CONTENT IN BALTIMORE $PM_{2.5}$ SEAS SAMPLES IS NOT SOLELY RESPONSIBLE FOR CHEMOKINE OR CYTOKINE RESPONSE IN A549 ATII CELLS OR RAW 264.7 MONOCYTES.

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For approximately a decade, metals have been hypothesized to contribute to the relationship between human exposure to fine particulate matter ($PM_{2.5}$) and cardiopulmonary disease. Recently, the metal zinc has been shown to play a pivotal role in inducing cardiac lesions and inflammation *in vivo* (Kodavanti *et al.* 2003). In order to test the hypothesis that zinc plays a central role in the release of soluble inflammatory mediators in the lung, we exposed human alveolar type II cells (A549) and monocytic cells (RAW 264.7) to fine particulate matter ($PM_{2.5}$; SEAS samples) collected at the Baltimore Supersite during summer and winter intensive sampling periods in 2002. Median TNF-α levels from RAW 264.7 monocytes were greater following treatment with the November $PM_{2.5}$ samples, while MCP-1 release from A549 alveolar epithelial cells was significantly inhibited by the November samples, compared to the July $PM_{2.5}$. A comparison of eleven metal concentrations measured by GFAAS revealed that Al, Fe, and Zn were the most abundant in both the July (Fe>Al>Zn) and November (Fe>Zn>Al) samples. Multiple linear regression revealed that Zn in combination with endotoxin, Cd, Fe, and particle number explained 50% of TNF-α release in November samples, while particle number and Fe explained approximately 44% of the inhibition of MCP-1 release in this same sampling period. Neither zinc, nor any other metal alone, was responsible for the release of the immune mediators. These results challenge prevailing research paradigms that view one or more metals as being solely responsible for the adverse effects of inhaled $PM_{2.5}$ and demonstrate the need for more complex statistical approaches to aid in determining the relative contributions of the various components of $PM_{2.5}$, as well as particle number, to cardiopulmonary inflammation. *Supported by the Baltimore Supersite Program grant R82806301*

1452 EFFECT OF PULMONARY EPITHELIAL LINING FLUID ON OXIDATIVE STRESS AND DNA DAMAGE: COMPARISON AMONG ULTRAFINE PARTICLES, FERROUS SULFATE AND DIESEL PARTICLE EXTRACT.

T. Cheng, Y. Cheng and Y. Lei. *College of Public Health National Taiwan University, Taipei, Taiwan.* Sponsor: T. Ueng.

Previous studies have reported that particulate matter can induce oxidative stress and DNA damage. To protect lung cells against the exogenous oxidative stress, pulmonary epithelial lining fluid (ELF) consists of various antioxidants. However it is not clear which components of PM are affected by ELF. The aim of this study is to determine the effect of ELF on oxidative stress and DNA damage induced by several PM components including ultrafine particles, ferrous sulfate and diesel particle extract. In cell free system, ultrafine carbon black of 15 nm (ufCB; 0, 50, 150μg/ml), FeSO4 (0, 100, 500μgM), and diesel particle extract (0, 250, 500μg/ml) were suspend in ELF or medium. Subsequently, we used transwell *in vitro* culture system with ELF or medium on the top of A549 cells and medium below. Again, similar concentrations for each component were used. After 4 hr of treatment, ROS was then measured using DCFH assay and DNA single strand breakage was also determined by single-cell gel electrophoreses (Comet assay). The results showed that in cell free system and A549 cells with culture medium, ROS increased with exposure to ufCB and FeSO4, but not with diesel particle extract. We also observed DNA SSB increased with FeSO4 and diesel particle extract exposure in culture medium, but not with ufCB. When ELF was used to replace medium, ROS decreased significantly for both ufCB and FeSO4 exposure in cell free and A549 cells. There was no increase for DNA SSB for each component in ELF. We conclude that ELF may decrease ROS levels and subsequent DNA SSB formation induced by ufCB and FeSO4.

1453 EFFECTS OF PM-ASSOCIATED METALS ON MACROPHAGE INOS AND ERK: A ROLE IN ALTERED IRON HOMEOSTASIS?

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Analyses of fine airborne $PM_{2.5}$ samples collected in New York City (NYC), Los Angeles, and Seattle during Fall 2001 indicated that inorganic composition varied from city to city, both in terms of absolute amounts of individual elements and their mass relationships to one another. We hypothesized that relative molar/mass relationships between select PM metals and Fe governs the pulmonary immunotoxic potential of a given day's (or city's) PM by affecting alveolar macrophage (AM) iron status, thereby altering their antibacterial function. To test this, iron response protein (IRP) binding activity in NR8383 rat AM was assessed after exposure to Fe alone or in combination with V, Mn and/or Al. In all studies, relevant molar ratios of the metals, as found in a representative 500 μg sample of NYC PM, were used. Results indicated that V and Al, and to a lesser extent Mn, significantly changed IRP activity though the effects were not consistently dose-dependent. This disruption in IRP binding activity was thought to possibly be a result of competition for binding to a transferrin carrier; however, potential effects on IRP activity from the presence of nitric oxide (NO) needed to be ruled out. The results of analyses of concurrent expression of iNOS and phospho-ERK1/2 suggested that while V treatments did not activate NO formation, Al did - most likely via increases in lipid peroxidation that resulted in AP-1 activation. These results confirm that certain metals associated with PM might alter pulmonary immune competence in exposed hosts by impacting upon the Fe status of one major class of deep lung defense cell. This work was supported by EPA/PM Center Grant R82735101.

1454 MUTAGENICITY AND CYTOXICITY OF MANUAL METAL ARC STAINLESS STEEL WELDING FUMES (MMA-SS) BY ITS PARTICLE SIZE.

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The particle size and metal components of fine particulates have been hypothesized to be important factors in determining in their toxicities and potential adverse health effects. To study cytotoxicity and mutagenicity of MMA-SS by their particle

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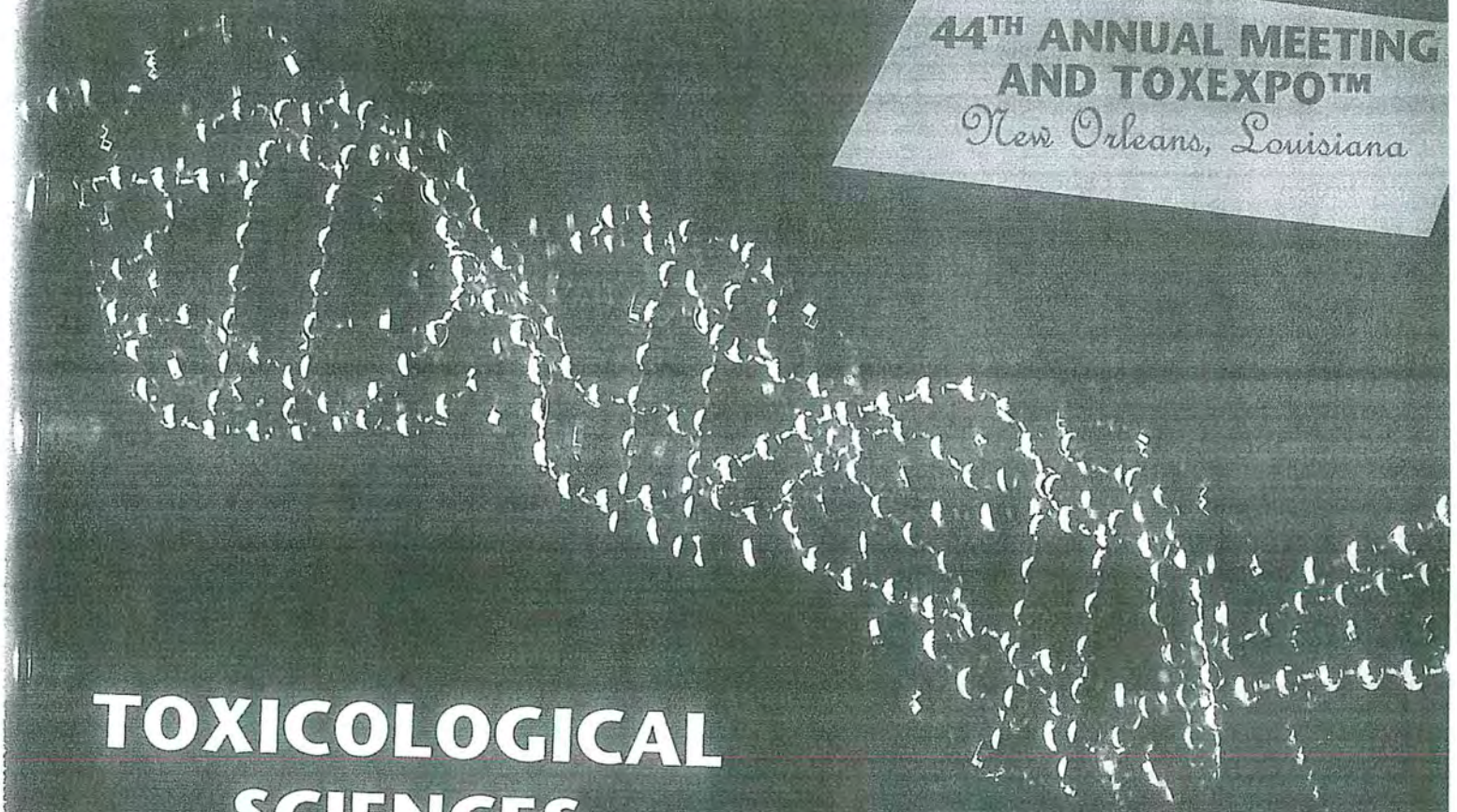

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