lung ELF GSH levels. Cystic fibrosis (CF) patient have low levels of GSH in their ELF and have copious and viscous mucus. Hypertonic saline is used in CF patient to help clear mucous and improve lung function however the mechanism(s) by which it does so are poorly understood. The purpose of these studies was to examine whether hypertonic (3.0%) saline can modulate apical GSH levels and protect lung epithelial cells against the oxidant, t-butyl hydroperoxide (t-BOOH). We used human lung epithelial cell lines sufficient (C38) and deficient (IB3) in CFTR. The CFTR deficient IB3 cells have 40% lower basal levels of apical GSH as compare to CFTR sufficient C38 cells. Cells were exposed separately and in combination to 3% saline, and t-BOOH (100 μM) for 48 hours. The CFTR deficient IB3 cells were much more sensitive to t-BOOH-mediated oxidative injury as measured by lactate dehydrogenase (LDH) release. Hypertonic saline exposure was associated with an increase in apical GSH levels in both CFTR sufficient and deficient cells and decreased t-BOOH-mediated oxidative injury in both cells lines. Hypertonic saline increased GSH in the apical compartment, which appeared to be largely CFTR mediated. This data suggests that CFTR and GSH adaptive responses play an important role in lung's reaction to oxidants. We propose that factors, which interfere with the lung's capability to mount and maintain an adequate adaptive apical GSH response, may compromise and sensitize the lung to oxidative injury. (Supported in part from NIH grant HL075523).

182 DIFFERENTIAL MODULATION OF STRESS SIGNALING PATHWAYS BY CADMIUM IN CULTURED MOUSE LUNG FIBROBLASTS HETEROZYGOUS FOR GPX4.

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Cadmium is a widely dispersed environmental and occupational toxicant and has been associated with mutagenic, carcinogenic, teratogenic and neurotoxic endpoints. Inhalation exposure to cadmium has been associated with airway inflammation, pulmonary edema, emphysema, and cancer. Cadmium causes oxidative stress in cells by formation of reactive oxygen species (ROS), induction of lipid peroxidation, binding to protein thiols, and alteration of intracellular glutathione status. Glutathione peroxidase 4 (GPx4) is a member of the family of selenium dependent enzymes that catalyze the reduction of peroxides. GPx4 specifically reduces mitochondrial membrane-bound phospholipid hydroperoxides in situ and thus, protects against membrane peroxidative damage. Mouse lung fibroblasts deficient in GPx4 have increased susceptibility to cadmium-induced cytotoxicity. In the present study cultured mouse lung fibroblasts from wild-type (+/+) and GPx4 heterozygous (+/-) mice were exposed to cadmium chloride (CdCl2), and cell extracts were analyzed for stress (p38, JNK) and survival (Akt) pathway activation by Western blot analyses. Levels of phosphorylated p38 and JNK were increased following CdCl2 treatment in GPx4+/+ cells, but less so in GPx4+/- cells. Phosphorylated Akt levels, though decreased slightly, remained high in GPx4+/+ cells, but dropped to only 10% or less of control in GPx4+/- cells. However, GPx4+/- cells exhibited an attenuated survival response, as measured by the ratio of survival pathway activation (Akt) to stress pathway activation (p38 and JNK). These results implicate the involvement of survival and stress signaling pathways in cadmium-induced cytotoxicity, and the potential involvement of these pathways in defining the differential susceptibility to cadmium-induced stress in cells deficient in GPx4.

183 DIESEL EXHAUST PARTICLE-INDUCED OXIDANT INJURY AND CELLULAR RESPONSES IN WILD TYPE AND INDUCIBLE NITRIC OXIDE SYNTHASE-DEFICIENT (INOS KO) MICE: ROLES OF PARTICLE CORE AND ADSORBED ORGANICS.

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Studies have shown that diesel exhaust particle (DEP) exposure induces lung inflammation and injury, which are mediated through reactive oxygen/nitrogen species (ROS/RNS) generation by alveolar macrophages (AM). The present study examines the differential roles of DEP particle core, represented by carbon black (CB), and DEP organic extract (DEPE) in ROS generation and nitric oxide (NO) production through inducible nitric oxide synthase (iNOS)-related cellular responses using C57B/6J wild type (WT) and iNOS knockout (iNOS KO) mice. Mice (8-10 weeks old) were exposed to saline, CB (35 mg/kg), or DEPE by aspiration and sacrificed at 1, 3 and 7 days post-exposure. AM were isolated by bronchoalveolar lavage (BAL). CB, but not DEPE, significantly induced neutrophil infiltration in both WT and iNOS KO mice. CB-exposed AM also exhibited enhanced hydrogen peroxide and superoxide anion generation, peaking at 3 d post exposure. This CB-induced oxidative stress was correlated with mitochondrial damage, involving reduction of mitochondrial mass and membrane potential in AM from both WT and iNOS KO mice. These results suggest that NO did not play a major role in modifying CB-induced mitochondrial damage in AM. In contrast, the organic component of DEP, DEPE, only slightly induced ROS generation and did not significantly affect mitochondrial function in AM. Measurement of macrophage ATP content showed that CB and DEPE did not significantly affect ATP levels in either WT or iNOS KO mice. This is due to the fact that mitochondrial oxidative phosphorylation pathway is not the major energy source for AM. In summary, this study shows that CB, but not DEPE, plays a major role in DEP-induced pulmonary inflammation and AM mitochondrial dysfunction by a mechanism independent of NO. (The findings and conclusions in this abstract have not been formally disseminated by the NIOSH and should not be construed to represent any agency determination or policy.)

184 BLOCKADE OF HMGB1 INHIBITS HYPEROXIA-INDUCED PRO-INFLAMMATORY LUNG INJURY.

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Prolonged exposure to hyperoxia results in acute lung injury (ALI) with markedly elevated levels of proinflammatory cytokines and infiltrated leukocytes in lungs. However, the mechanisms underlying hyperoxia-induced proinflammatory ALI remain poorly understood. Here we report our studies on determining the role of high mobility group box protein 1 (HMGB1), a newly discovered proinflammatory cytokine, in hyperoxic lung injury. Exposure of adult mice to >99% oxygen increased the accumulation of HMGB1 in the bronchoalveolar lavage fluid, which preceded the onset of severe lung injury. Neutralizing anti-HMGB1 antibodies, administrated to mice 24 hours prior to hyperoxic exposure, significantly mitigated ALI, indicated by the levels of the wet/dry ratio, lung permeability and total leukocyte infiltration. This protection was also observed when the treatment with HMGB1 inhibitors was delayed until after the onset of the oxidative stress. In addition, ethyl pyruvate, a stable aliphatic antioxidant, not only inhibited HMGB1 secretion from hyperoxic macrophages, but also mitigated hyperoxic lung injury when administrated post hyperoxic exposure. The contribution of HMGB1 to the initiation of hyperoxic lung injury was further confirmed using purified recombinant HMGB1 (rHMGB1) protein instilled intratreacheally. Administration of rHMGB1 caused a marked increase in the levels of leukocyte infiltration into the lung of these mice compared to those that were treated with the same amounts of non-specific peptide ($31\pm4.8x10^4$ vs. $14\pm3.4x10^4$, $p<0.05$). Taken together, these results indicate that HMGB1 plays a critical role in mediating hyperoxic lung injury through the recruitment of leukocytes into the lung, and support the potential clinical application of HMGB1 inhibitors as therapeutic interventions against oxidative lung injury in patients receiving hyperoxia.

185 METABONOMIC SIGNATURES OF SILICA NANOPARTICLE RESPIRATORY TRACT TOXICITY IN NORMAL AND DISEASED ANIMALS.

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Characterizing the hazard potential of the significant number of nanomaterials that have been or will be produced is one of the most noteworthy challenges faced by the regulatory, research, and producer communities. Ultrafine (aerodynamic diameter dae, 0.1 μm) and fine (dae, 2.5 μm) particulate matter is associated with decreased lung function in child and adult asthmatic populations and increased cardiovascular morbidity and mortality in the general population. One weakness of occupational studies is they generally do not consider effects on sensitive populations. The Wistar-Kyoto (WKY) derived spontaneously hypertensive (SH) rat is a useful model of cardiovascular disease with underlying chronic pulmonary injury, inflammation, and oxidative stress. Metabonomic analysis involves the quantitation of the multivariate response of an organism to a pathological event. To provide a metabonomic comparison of the biological response to nanoparticle exposure between the normal (WKY) and sensitive (SH) rat, male rats were given a single dose of amorphous silica (16 nm) at either 0.12 or 5 mg/kg via intratracheal instillation. Bronchoalveolar lavage fluid (BALF) and blood were collected 24 hours after exposure. Screening of small molecule biomarkers less than 1400 atomic mass units was accomplished using LC-ESI-MS. Comparison of peak areas from spectra of blood and BALF samples showed clear differences in endogenous levels of compounds associated with oxidative stress between nanoparticle exposed and control animals of both strains. More subtle changes in peak areas of endogenous compounds were observed in comparison of BALF and blood collected from hypertensive versus normotensive animals. This study indicates that non-invasive monitoring of readily available biofluids using mass spectrometry holds promise as a rapid methodology for tracking metabonomic changes and identification of biomarkers of nanomaterial induced respiratory and cardiovascular toxicity.

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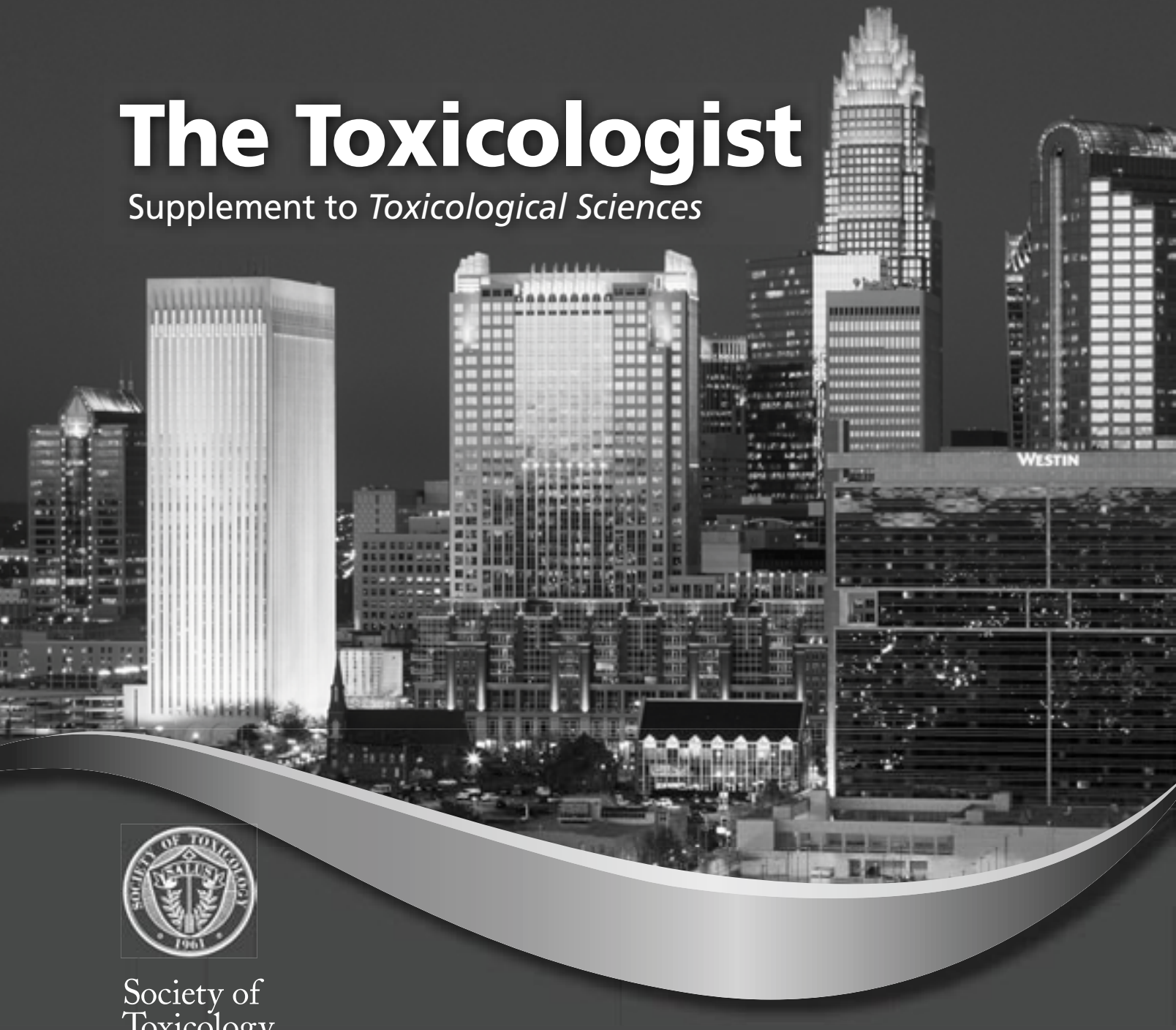

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 46th Annual Meeting of the Society of Toxicology, held at the Charlotte Convention Center, Charlotte, March 25–29, 2007.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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