AM and Type II cells constitutively expressed SOD and HO-1 mRNA. Although SOD mRNA levels were not significantly altered by O_3, HO-1 mRNA decreased over time in AM, but not in Type II cells. Similar results were observed with SOD and HO-1 protein expression. PPAR-γ, a nuclear hormone ligand activated transcription factors, has been reported to reduce lung injury associated with inflammation. Both AM and Type II cells constitutively expressed PPAR-γ mRNA. O_3 caused a transient decrease in PPAR-γ mRNA in AM, but a rapid increase in Type II cells. Taken together, our data demonstrate that O_3 inhalation results in augmentation of genes mediating the inflammatory response. Moreover, both AM and Type II cells produce mediators that help to limit the inflammatory response and maintain normal lung homeostasis. Support: ES004738, ES005022, ALA-NJ.

491 EXPOSURE TO GASOLINE ENGINE EXHAUST CAUSES OXIDATIVE STRESS IN RATS.

J. Seagrave[1], M. J. Campen[1], S. Dunaway[1], G. Herbert[1], J. L. Mauderly[1], J. D. McDonald[1] and A. C. Rohr[2]. *[1]Lovelace Respiratory Research Institute, Albuquerque, NM and [2]Electric Power Research Institute, Palo Alto, CA.*

Oxidative stress is a possible mechanism for some of the adverse health effects attributed to air pollution exposure. Some effects have been reported to be greater in populations living adjacent to heavily traveled roads, consistent with the hypothesis that fresh vehicle emissions may contribute disproportionately to the hazard. Diesel engine exhaust has been widely studied, but gasoline engine exhaust (GEE) has received much less attention. We used in situ chemiluminescence to assess the effect of inhalation of GEE in rats. This technique measures the decay to ground state of singlet O_2, a highly reactive form of O_2. Rats were exposed sequentially to exhaust from two 1996 General Motors 4.3 L gasoline engines, each run for 3h (total 6h) on a cycle simulating the Unified Driving Cycle 6. This protocol thus included two "cold starts." Rats were exposed to diluted GEE containing 60 μg/m^3 (average) of total particulate matter (TPM), to similarly diluted emissions passed through a HEPA filter to remove the particles, or to filtered air as a control. TPM, NOx, CO, and total hydrocarbons were measured. Following exposure, the rats were immediately removed from the exposure chambers, deeply anesthetized, and placed on a ventilator. The lungs, heart, and liver were exposed and covered with saline-saturated gauze and an opaque drape with a 1 cm diameter hole. The hole was aligned sequentially over the lungs, heart, and liver, and photon counting from each organ was performed using a high sensitivity photomultiplier tube and data acquisition system. The results showed a significantly higher signal in the organs (Lung > heart ≈ liver) of the rats that had been exposed to whole GEE, compared with those exposed to filtered air, but little effect of the filtered GEE. These results are consistent with the presence of increased reactive oxygen species due to inhalation of the particle phase of fresh GEE. Supported by the Electric Power Research Institute and the National Environmental Respiratory Center.

492 GASOLINE EMISSIONS AFFECT CLEARANCE OF INTRA-TRACHEALLY INSTILLED *PSEUDOMONAS AERUGINOSA.*

M. Reed, A. P. Gigliotti and J. A. Berger. *Toxicology, Lovelace Respiratory Research Institute, Albuquerque, NM.*

Gasoline emissions (GE) are contributors to ambient and occupational air pollution. Of the health outcomes associated with ambient pollutant exposures, respiratory infection is a major contributor to morbidity and mortality. As part of a larger health assessment of multiple anthropogenic source emissions, mice were exposed to GE and instilled with an inoculum of a common respiratory pathogen, Pseudomonas aeruginosa. GE was generated from two mid-mileage 1996 4.3 L GM engines operated on a simulated version of the Unified Driving Cycle 6 and fueled by national average unleaded gasoline. Groups of young C57Bl/6 mice were exposed to clean air, whole GE diluted to 18 (High), 12 (Mid), and 2 (Low) ppm oxides of nitrogen (NOx), and a particulate matter (PM)-filtered level (18 ppm NOx). PM concentrations were ~60, 30 and 7μg/m^3 at the High through Low levels respectively. Exposures were conducted for 6h/d for 7 consecutive days. Mice were instilled with bacteria subsequent to the last exposure and sacrificed after 18h in clean air. Pulmonary inflammatory histopathology and colony counts of serially diluted lung homogenate were assessed. GE had no discernable effect on exacerbation of pulmonary pathology. Similar to observations with diesel emissions, GE decreased bacterial clearance in an exposure-dependent manner. PM filtration partially attenuated the response. These results indicate that resistance to respiratory bacterial infection may be modulated by multiple on-road sources.
This abstract is funded by the National Environmental Respiratory Center (NERC) with support of multiple government and industry sponsors including the US EPA. This work is not intended to represent the view of any NERC sponsor.

493 CYTOGLOBIN IMMUNOLOCALISATION IN LUNG PARENCHYMA IN SEVERE COPD.

M. Graham[1], M. Carpenter[2], N. Hodges[2], K. Chipman[2], K. Pinnion[1] and M. Foster[1]. *[1]Safety Assessment, AstraZeneca, Leicestershire, Leicestershire, United Kingdom and [2]School of Biosciences, Birmingham University, Birmingham, United Kingdom.*

Cytoglobin is a novel member of the globin family that has a putative role as a tissue oxygen sensor. It is expressed in fibroblasts and has been shown to be an inducer of fibrosis, raising the possibility of a role in the pathogenesis of fibroproliferative toxicity and disease. In this study, we profiled cytoglobin expression in a cohort of patients with severe COPD undergoing lung volume reduction surgery, in which alveolar fibrosis and loss of vascularity are a feature. Ethically acquired COPD lung tissue was classified according to the fibroproliferative phenotype: pneumonitis (P), diffuse fibrosis (DF), marked alveolar fibrosis without loss of septation (AF) and organising fibrosis with loss of normal lung parenchyma (OF). In lung areas presenting with P, there was marked cytoglobin staining of macrophages, predominately a vesicular staining pattern suggestive of protein phagocytosis. There was little signal in adjacent areas. In DF areas, there was a diffuse signal within the interstitium, within fibroblasts and occasional mononuclear cells. As fibrosis increased from AF to OF, there was a progressive loss of cytoglobin signal from the interstitium, an increase in signal within the pulmonary vasculature and an increase in signal within pan-cytokeratin positive cells bordering areas of consolidated matrix – although typical epithelium within the conducting airways showed only a weak signal or negative signal. Additionally, a marked cytoglobin signal seen in the pulmonary vasculature was associated with vessels surrounded with fibrosis. In conclusion, cytoglobin expression in a disease cohort showing fibroproliferation and loss of vascularity shows a progressive loss of signal as the lesion matures to a more acellular phase, or where vascular structures are lost or surrounded by fibrosis. The histologically significant finding of cytoglobin expression in the peri-fibrotic cytokeratin positive cell colony raises the possibility that this expression may be an attempt by normal lung to limit the spread of hypoxic stimuli.

494 THE USE OF A FLOW CYTOMETRY-BASED CYTOKINE ANALYSIS FOR BRONCHOALVEOLAR LAVAGE WITH CONFIRMATION BY REAL-TIME RT-PCR IN A MOUSE MODEL OF PULMONARY INFLAMMATION.

P. C. Zeidler-Erdely, A. D. Erdely, S. Young and J. M. Antonini. *Health Effects Laboratory Division, NIOSH, Morgantown, WV.*

Bronchoalveolar lavage (BAL) provides a valuable tool for insight into the lung inflammatory state following a toxic or antigenic exposure. Toxicological injury evaluation routinely uses a concentrated first fraction BAL (<600 μl) to accurately measure various lung inflammatory parameters in mice. However, 100-250 μl of this BAL fraction is typically used in most conventional assays limiting the researcher to only a few measurements. Recently, flow-cytometry based cytometric bead arrays (CBA) have been developed to simultaneously determine concentrations of multiple analytes in a single 25-50 μl sample saving both cost and time. In this confirmatory study, we used a mouse inflammation CBA kit to analyze IL-6, IL-10, IL-12p70, MCP-1, IFN-γ, and TNF-α protein in BAL fluid obtained from A/J or C57BL/6J mice exposed to welding fume. Since inflammatory mediators are usually accompanied by both an increase in gene expression and protein, real-time RT-PCR was done on isolated RNA from whole lung homogenates to validate the findings. It was found that a limitation of the CBA kit was sensitivity. In particular, samples with low concentrations of an analyte were often reported as extrapolated below sensitivity or zero thus limiting statistical analysis. These findings made validation by some other method necessary. Real-time RT-PCR strongly supported the findings of the CBA kit by displaying similar trends in the mouse lung inflammatory response caused by welding fume exposure. Therefore, the mouse inflammation CBA kit was an acceptable method for measuring cytokines in studies involving limited sample size and represents a cost efficient screening method for pulmonary inflammation. Also, it is recommended that another method such as RT-PCR or ELISA be performed if increased sensitivity is required.

495 COMPARISON OF LUNG INJURY AND INFLAMMATION AFTER REPEATED TREATMENT WITH WELDING FUMES COLLECTED FROM DIFFERENT WELDING PROCESSES.

J. M. Antonini and J. R. Roberts. *NIOSH, Morgantown, WV.*

Welding fumes are a complex mixture of different metals. Depending on the welding process, some welders may be exposed to fumes that contain vastly different metal profiles. The goal was to compare the lung response in rats after multiple

treatments with welding fumes that are vastly different both chemically and physically. Welding fumes were collected from three different processes: gas metal arc-mild steel welding (GMA-MS); manual metal arc-hardsurfacing welding (MMA-HS); flux-cored arc-hardsurfacing welding (FCA-HS). Male Sprague-Dawley rats were treated by intratracheal instillation 1/wk x 7 wk with 0.5 mg/rat of the fume samples. Controls were treated with saline. Bronchoalveolar lavage was performed 4 days after the final treatment, and parameters of lung injury (lactate dehydrogenase and albumin) and inflammation (neutrophil influx) were assessed. Metal analysis indicated that the GMA-MS fume was primarily composed of Fe (1.08 μg Fe/gm total metal) and Mn (0.32 μg Mn/gm total metal), whereas the Mn content of the FCA-HS (2.0 μg Mn/gm total metal) and MMA-HS (1.8 μg Mn/gm total metal) fumes was ~6x higher than in the GMA-MS fume. The FCA-HS (0.07 μg Cr/gm total metal) and MMA-HS (0.304 μg Cr/gm total metal) fumes contained Cr which was present in only trace amounts in the GMA-MS fume. The FCA-HS and MMA-HS fumes were found to be more water-soluble than the GMA-MS fume. Significant elevations in lactate dehydrogenase, albumin, and the number of lung neutrophils were observed for the MMA-HS and FCA-HS groups compared to the GMA-MS and saline groups. No significant differences in lung injury and inflammation were observed between the GMA-MS and saline group. The greater lung response caused by the FCA-HS and MMA-HS fumes is likely due to the presence of Cr and higher levels of Mn. Results from this study indicate that welders who are exposed to fumes generated from specific processes may be at a greater risk for adverse pulmonary effects.

496 TOXICOKINETICS OF SURFACTANT PROTEIN AND INFLAMMATORY GENE EXPRESSION IN PRIMARY RAT LUNG ATII CELLS AND FIBROBLASTS EXPOSED TO PENICILLIUM CHRYSOGENUM AND STACHYBOTRYS CHARTARUM TOXINS.

T. G. Rand[1], A. Kading[1], J. D. Miller[3], J. E. Scott[2] and C. Robbins[1]. *[1]Biology, Saint Mary's University, Halifax, NS, Canada, [2]Department of Oral Biology Anatomy, University of Manitoba, Winnipeg, MB, Canada and [3]Chemistry, Carleton University, Ottawa, ON, Canada.* Sponsor: J. Pestka.

Not a great deal is known about the effects and modes of action of the spores and toxins of the majority of molds encountered in the indoor environment on lung biology. In vivo models of lung disease indicate that fungal spore and toxin exposures lead to a variety of species and toxin-specific inflammatory responses. However, it is unclear whether all the lung cells participate in these inflammatory changes. In this study we used gene expression profiling using custom made RT-PCR based microarrays to compare surfactant protein and inflammatory gene responses in primary fetal rat lung ATIIs and fibroblasts to atranones A&C from Stachybotrys chartarum (Sc) and melagrin and roquefortine C from Penicillium chrysogenum (Pc). In Pc-toxin exposed ATIIs, inflammatory genes were transiently up-regulated, generally in dose and time dependent ways, within 2 to 4 hr and de-expressed by 12 hr post exposure (PE), especially in meleagrin exposed cells. SP-A,-B &-C genes were down regulated at 4 hr PE. SP-D expression was unaffected by the Pc toxin exposures. Inflammatory genes were generally up-regulated in atranone A but down regulated in atranone C exposed ATIIs from 2 to 24 hr PE, and in a dose dependent fashion. SP-A & -C genes were down regulated in both atranone A & C exposed cells, while SP-B & -D expression was unaffected. In fibroblasts, gene expression was generally limited to SP-D up-regulation, while MIP-3α was down regulated, from 2 to 4 hr PE. The results indicate that toxins from two mold species common on damp materials in buildings provoke compound-specific toxic responses with different toxicokinetics. They also provide further insight into molecular mechanisms of spore and toxin induced lung disease onset and development, and may be useful in the identification of fungal specific molecular biomarkers of disease.

497 REPEATED EXPOSURE TO 1→3-β-GLUCAN SUPPRESSES LUNG DEFENSE RESPONSES AND SLOWS THE PULMONARY CLEARANCE OF LISTERIA MONOCYTOGENES IN RATS.

S. Young, J. R. Roberts and J. M. Antonini. *NIOSH, Morgantown, WV.*

Chronic exposure to low levels of mold has been reported to increase susceptibility to respiratory infections. Currently, the mechanism is not well understood. This study investigated lung defense effects after repeated low-dose zymosan (a 1→3-β-glucan from yeast cell wall) exposure on bacterial infection (Listeria monocytogenes) in male Sprague-Dawley rats. On days 0, 3, 7 and 10, rats received one dose of zymosan A (0.6 mg/kg body weight each dose) via intratracheal instillation or vehicle control (saline). On day 17 (one week later), rats were intratracheally inoculated with $5x10^5$ bacteria. Rats were euthanized on days 20, 22, and 24. Bacterial clearance was determined by measuring colony-forming units cultured from the left lungs. Bronchoalveolar lavage (BAL) was performed on the right lungs. Inflammation and lung injury were assessed by measuring (1) neutrophil (PMN) infiltration and (2) albumin and lactate dehydrogenase levels in BAL fluid. Multiple zymosan treatments induced greater lung injury and inflammation as indicated by elevations in PMN, LDH, and albumin at days 22 and 24 compared to control. In addition, repeated low-dose exposure to zymosan slowed the clearance of the bacteria at day 20 and 22 compared to controls, suggesting a possible suppression in lung immune responses. In contrast, previous results have shown that a single acute dose of zymosan at a concentration (2.5 mg/kg body-weight) equivalent to the total of the four repeated concentrations used in the current study enhanced lung immune responses and increased the rate of bacterial clearance from the lung. This study demonstrated the importance of treatment concentration and dosing regimen in understanding lung immune effects associated with 1→3-β-glucan exposure.

498 90 DAY INHALATION TOXICITY STUDY WITH DISK-SHAPED POTASSIUM OCTATITANATE PARTICLES (POT; TERRACESS TF) IN RATS.

S. Sakai[1], A. K. Tanaka[1], D. P. Kelly[2], G. Sykes[3] and K. P. Lee[4]. *[1]Otsuka Chemical Co., Ltd., Osaka, Japan, [2]Haskell Laboratory, DuPont Company, Newark, DE, [3]PharmPath, West Grove, PA and [4]Pathology Consulting, Newark, DE.* Sponsor: S. Loveless.

Fibrous particles such as asbestos and some man-made fibers have been known to produce carcinogenic or fibrogenic effects upon inhalation. Non-fibrous, disk-shaped potassium octatitanate particles (POT; Terracess TF) have been manufactured to avoid potential fiber toxicity. Four groups of 20 male and 15 female rats each were exposed to POT aerosol at concentrations of 0, 2, 10, or 50 mg/m3 for a 90 day period followed by a 15 week recovery period. The mass median aerodynamic equivalent diameter of the aerosols ranged from 2.5 to 2.9 μm. Lung burdens of POT were determined in 5 males/group at the end of the exposure period and after 3 and 15 weeks recovery. The clearance ½ times for POT were estimated to be in the order of 2 to 3 months for the 2 and 10 mg/m3 groups and 6 to 9 months for the 50 mg/m3 group. There were no POT related adverse effects in clinical observations, body weights, clinical pathology, or neurobehavioral measurements. At the end of the 90 day exposure, a slight increase in lung-to-body weight ratios was observed at 50 mg/m3 in males; this effect was not seen during the recovery periods. Female lung weights were normal. Microscopically, inhaled POT particles were mostly phagocytized by alveolar macrophages (AMs) in the alveoli and alveolar walls maintained their normal structure at 2 and 10 mg/m3. At 50 mg/m3, some alveoli were distended and filled with aggregates of particle laden AMs. The alveolar walls showed slight Type II pneumocyte hyperplasia but neither active inflammation nor alveolar fibrosis was present. The lung responses and lung clearance rates of POT were comparable to those of "nuisance" type dusts at these concentrations. The slower clearance rates at 50 mg/m3 and the presence of aggregated particle-laden AMs in alveoli at this level were considered signs of alveolar "overloading" and 50 mg/m3 was considered an effect level; the NOAEL was 10 mg/m3.

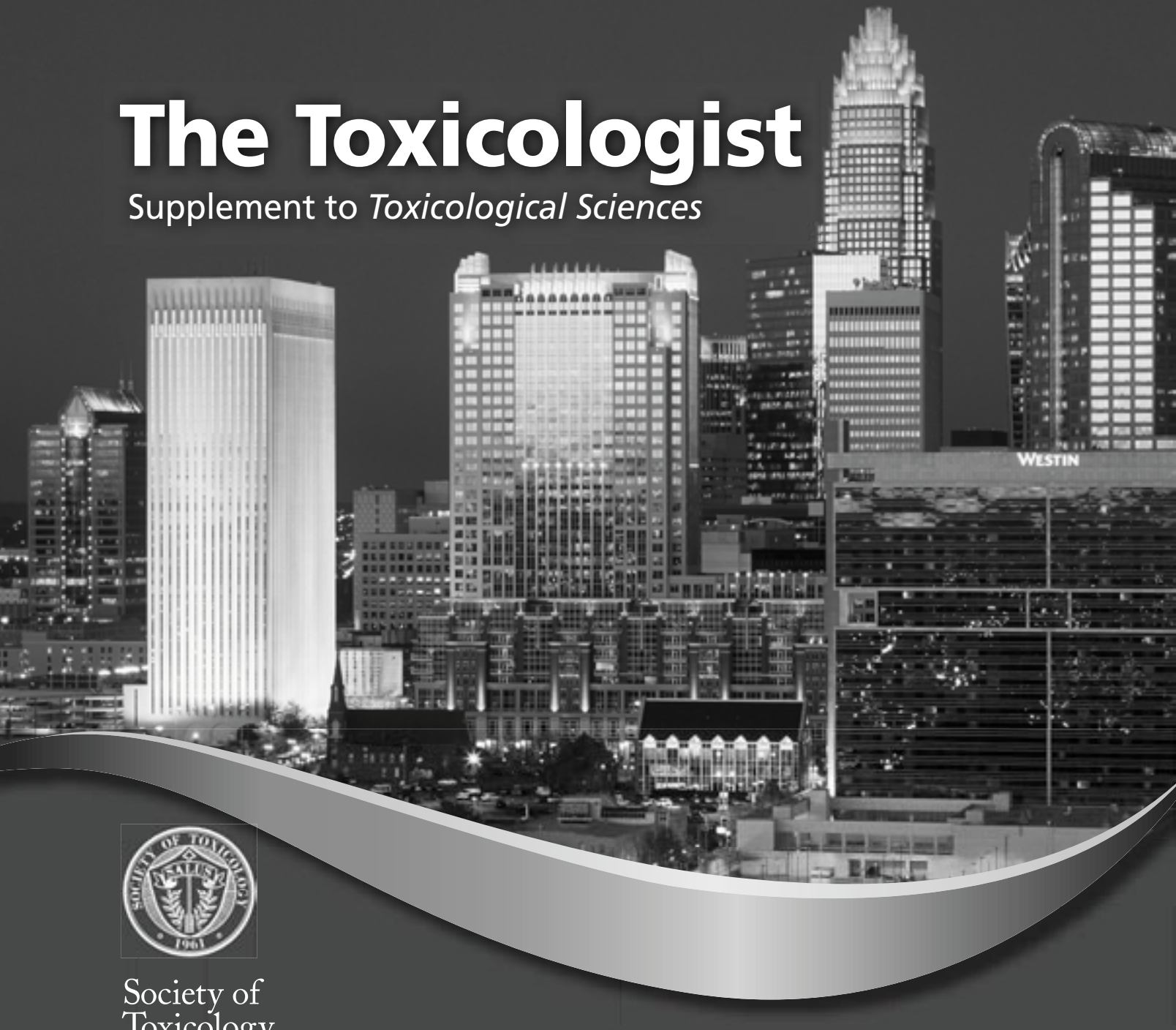
499 ESTABLISHMENT OF A BIOASSAY FOR DETECTION OF LUNG TOXICITY DUE TO FINE PARTICLE FROM BASED ON RESULTS OF DOSE RESPONSE STUDY.

M. Yokohira[1], K. Yamakawa[1], K. Hosokawa[1], Y. Matsuda[1], K. Saoo[2], T. Kuno[1] and K. Imaida[1]. *[1]Onco-Pathology, Department of Pathology and Host-Defence, Faculty of Medicine, Kagawa University, Kita-gun, Kagawa, Japan and [2]Department of Clinical and Pathological Laboratory, Kaisei General Hospital, Sakaide-Shi, Kagawa, Japan.* Sponsor: S. Tomoyuki.

In order to establish an appropriate bioassay for detection of lung damage after fine particle expose, sequential histopathological changes were examined after intratracheal instillation to quartz, as a typical lung toxic agent, into F344 male rats.
Experiment 1: A total of 60, 10-week-old male F344 rats, were randomely separated into 6 groups and each group was consisted 10 rats. Groups 1-4 were exposed to 4mg, 2mg, 1mg and 0mg (control) quartz (DQ-12) suspended in saline (0.2ml) using an aerolizer and subgroups were sacrificed 5 rats on Days 1 and 28 thereafter. Groups 5 and 6 were exposed to 4mg and 0mg (air only) quartz powder without suspension using special aerolizer and sacrificed on the same days. All groups underwent assessment of lung histopathology.
Experiment 2: A total of 72, 10-week-old male F344 rats, were randomly separated into 6 groups. 60 rats (5 groups) were exposed by intratracheal instillation to quartz, titanium dioxide, hydrotalcite and β-cyclodextrin, 2mg/rat, suspended in 0.2ml saline (established by Experiment 1) and subgroups of 6 rats were sacrificed on Days 1 and 28. 12 rats were exposed by intratracheal instillation to 0.2ml saline as control groups, remaining 12 rats were not exposed as untreated group and subgroups of 6 rats were similarly sacrificed on the same days. All groups underwent assessment of lung histopathology and immunohistochemical demonstration of BrdU and iNOS as end-point markers.
From Experiment 1, 2mg quartz suspended in 0.2ml saline were suggested to be most appropriate for sensitive detection of inflammatory changes. By histopathology and immunohistochemical assessment, the level of toxicity were considered

The Toxicologist

Supplement to *Toxicological Sciences*



Society of Toxicology

46th Annual Meeting and ToxExpo™

Charlotte, North Carolina

An Official Journal of the Society of Toxicology

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 96, Number 1, March 2007

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.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org