

**PS 1193 Recurrent DNA Damage Indicates Persistent Injury in Progressive Radiation-Induced Pulmonary Fibrosis**

T. Beach, C. J. Johnston, A. M. Groves, J. P. Williams, and J. N. Finkelstein. *University of Rochester, Rochester, NY.*

Radiation injury is a clinically relevant outcome of radiotherapy for cancer treatment. Radiation-induced lung injury (RILI) occurs in some 40% of patients receiving radiation treatment for many forms of lung cancer (in a dose- and volume-dependent manner). Even though animal models and data from human patients with RILI have existed for decades, very little progress has been made in the treatment of these often-fatal side effects of radiotherapy. Traditionally, radiation treatment has been thought to produce DNA damage as a direct effect of radiation exposure, and that later effects of RILI are resultant of the injury and cell death sustained during radiotherapy/radiation treatment. Utilizing a well-established mouse model of radiation-induced pulmonary fibrosis (RIPF), a late effect of radiation treatment, we will show that recurrent DNA damage and chronically elevated oxidative stress are characteristic of this persistent, progressive, and often fatal injury. Irradiated and age-matched unirradiated C57BL/6J female mice were given 12.5 Gy thorax-only gamma radiation treatment, and sacrificed at 24 hrs, and 1, 4, 12, 16, 24, and 32 weeks following radiation exposure. Whole-lung tissues were examined by RNA sequencing, qPCR, colorimetric assay, histology, and immunofluorescence for evidence of DNA damage ( $\gamma$ -H2A.X and p53bp1), cellular senescence (senescence-associated beta-galactosidase, and p21), and oxidative stress by malondialdehyde (MDA). These data reveal recurrent DNA damage, persistent cellular senescence, and oxidative stress up to 32 weeks after the initial radiation treatment. From this novel, long-term dataset it appears that the prevention of DNA damage, as well as mitigation of ROS production and activity, are potential strategies for the treatment or prevention of these persistent toxic, lethal effects of pulmonary radiation injury.

**PS 1194 Data Comparison of the Respiratory Parameters during the Acclimatization Phase of Minipig Inhalation Studies**

S. A. Moore, and E. L. Moore. *Envigo, Huntingdon, United Kingdom.*

The minipig is increasingly being used as a species of choice for toxicological and pharmacological studies due to improved animal welfare considerations and increased ethical scrutiny of using non-human primates. However, this is not the case for inhalation studies. Prior to the exposure phase of every inhalation study, the animals were acclimatized to the restraint mask procedure over a period of 20 days for up to 4hrs in duration. The aim was to minimize stress-related elevations in respiratory minute volume (RMV) values, which may mask potential effects of administered test material. Pre-dose measurements of tidal volume (TV), respiration rate (RR), and RMV were collated over 4 occasions from 3 males and 3 females (bodyweight range 25-30kg) to ascertain whether there were any differences between the recording sessions. The average RR on Day 8 was 25.4breaths/min, which decreased to 20.1 after Day 16. The average TV for Day 8 was 193mL/min, decreased to 142 on Day 16. This resulted in RMV (RRxTV) values for Day 8 and Day 16 of 5.26L/min and 3.03L/min, respectively. However, there was no statistical significance difference due to the animal to animal variability. The average values at Days 10 and 14 showed a general decrease in RR. The RR, TV, and RMV values for the females were higher than the males. Comparing the inhalation dose values with pre-dosing values (-1 to -0.67hr), the inhalation restraint procedure gave a marked average decreases in RR, TV, and RMV from 16.1breaths/min, 130mL/min, and 2.04L/min. These values markedly increased to 40.9breaths/min, 124mL/min, and 6.11L/min 5min prior to dosing. The values from 2 to 21hrs post-dose gave values of 13.3breaths/min, 132mL/min, and 1.73L/min at Day 16. Comparing the RMVs against the Alexander equation<sup>1</sup> during restraint procedures found that this dataset produced RMV values that were up to 70% than predicted based on the bodyweight, suggesting that this equation overestimates the RMV for minipigs and RMV values should be ascertained prior to every study. However, it must be noted that no equivalent data were used in that equation's derivation. In conclusion, the RR, TV, and RMV decreased with the number of acclimatized exposures (42%) but were considerably higher than observed pre- and post-exposure. The RMV of minipig is 70% lower than predicted by the Alexander et al RMV equation<sup>1</sup>, an RMV determination should be undertaken prior to every study, and a species-specific RMV equation is necessary.

**PS 1195 Altered Functional and Molecular Responses of Endothelial Cells Treated with Serum Collected from Rats Exposed to Different Welding Fumes**

J. M. Antonini, V. Kodali, M. Shoeb, T. G. Meighan, T. Eye, T. A. Stueckle, and A. Erdely. *NIOSH, Morgantown, WV.*

Epidemiological studies suggest an interplay between exposure to particulate matter and the rise in adverse cardiovascular outcomes. The precise mechanism of how pulmonary exposure translates to cardiovascular dysfunction is largely unknown. Recent studies suggest alterations in circulating factors as the primary cause of pulmonary-induced cardiovascular dysfunction. Because serum is composed of a myriad of discrete biochemical components, it is not only costly but selection affects the bias measurement of individual components. Because vascular endothelial cells play a pivotal role in cardiovascular disorders, we used serum collected from exposed animals to assess its molecular and functional effects on *in vitro* cultures of primary cardiac microvascular endothelial (PCME) cells or *ex vivo* cultured aortas from naïve rats as a strategy for evaluating altered endothelial cell function. This approach was validated using serum obtained from Sprague-Dawley rats at 24 h after intratracheal instillation of 2 mg/rat of different welding fumes (WF): manual metal arc welding using stainless steel electrodes (MMA-SS) and gas metal arc welding using mild steel electrodes (GMA-MS) or PBS (vehicle control). As an initial screen, PCME cells from rats were challenged for 4 h with serum from WF or vehicle-exposed animals, and 84 genes related to endothelial cell biology were analyzed from the mRNA isolated from challenged cells. In order to understand the functional and biological impact of the differentially expressed genes, Ingenuity Pathway Analysis (IPA) was performed. IPA predicted that serum from animals exposed to MMA-SS, and not GMA-MS or PBS, had influence on several functional aspects of endothelial cells, including angiogenesis and migration. These functional predictions were further validated with a scratch assay for migration (*in vitro*) and aortic ring assay for angiogenesis (*ex vivo*). As predicted, both functional endpoints were significantly changed when challenged with serum from MMA-SS but not GMA-MS, suggesting that pulmonary MMA-SS exposure has the potential to cause altered endothelial function systemically. This methodology can easily be adapted to a high-throughput screening platform and be utilized as a quick primary screening strategy for evaluating systemic cardiovascular toxicity due to various pulmonary exposures

**PS 1196 Oleic Acid Induces Acute Pulmonary Injury and Inflammation *In Vivo***

V. L. Bass<sup>1</sup>, M. C. Schladweiler<sup>2</sup>, U. P. Kodavanti<sup>2</sup>, A. J. Ghio<sup>2</sup>, and M. C. Madden<sup>2</sup>. <sup>1</sup>University of North Carolina at Chapel Hill, Chapel Hill, NC; and <sup>2</sup>US EPA, Research Triangle Park, NC.

Oleic acid (OA) is frequently used as a representative fatty acid, and is found in meat-cooking fumes and biodiesel exhaust. Lung and vascular injury has been observed with intravenous infusion of OA in acute respiratory distress models, but it is not yet established whether OA deposited in the lung is able to produce adverse pulmonary and cardiovascular effects associated with exposure to ambient air pollution. Iron has been demonstrated to attenuate lung injury induced by some air pollutants. We hypothesized that OA via intratracheal (IT) exposure would induce lung injury and alteration to vascular biomarkers, and that these changes could be mitigated by the co-administration of excess iron. Sixty-four Wistar Kyoto rats aged 12 weeks were assigned to one of four treatments: vehicle control (0.04% EtOH in saline), OA (20mg/kg), ferric ammonium citrate (FAC) (0.43mg/kg), or OA + FAC (20mg/kg OA + 0.43mg/kg FAC) administered IT (1ml/kg). Plethysmography and collection of tissues, bronchoalveolar lavage fluid (BALF), and blood was performed at 4hr or 24hr after instillation. OA increased enhanced pause (PenH), an index of air-flow limitation (saline control: 0.66±0.04; OA: 1.5±0.1; OA +FAC: 1.8±0.2). OA increased BALF LDH at 4hr, (saline control: 37.6±1.6 U/L; OA: 59.2±2.2 U/L; OA + FAC:87.6±5.3 U/L). BAL neutrophil count in the lung increased by 20.8±3.8 percent with OA and 27.3±5.5 percent with OA + FAC, relative to the control. Total BALF protein and albumin were significantly higher in OA (at 4 and 24hr) and were further exacerbated in OA + FAC rats at 4hr. FAC alone did not significantly alter BALF markers of lung injury and inflammation. Collectively, these data indicate that when given IT, OA induces acute lung injury and inflammation. Rather than mitigating lung damage when given simultaneously IT, excess iron in FAC appears to exacerbate these effects. *This abstract does not reflect official US EPA policy.*

# The Toxicologist

Supplement to  
*Toxicological Sciences*



57<sup>th</sup> Annual Meeting  
and ToxExpo™

San Antonio, Texas  
March 11–15, 2018

**OXFORD**  
UNIVERSITY PRESS

ISSN 1096-6080  
Volume 162, Issue 1  
March 2018

[www.academic.oup.com/toxsci](http://www.academic.oup.com/toxsci)

The Official Journal of  
the Society of Toxicology

**SOT** | Society of  
Toxicology

[www.toxicology.org](http://www.toxicology.org)

# The Toxicologist

Supplement to  
*Toxicological Sciences*



## 57<sup>th</sup> Annual Meeting and ToxExpo™

San Antonio, Texas  
March 11–15, 2018

**OXFORD**  
UNIVERSITY PRESS

ISSN 1096-6080  
Volume 162, Issue 1  
March 2018

[www.academic.oup.com/toxsci](http://www.academic.oup.com/toxsci)

The Official Journal of  
the Society of Toxicology

**SOT** | Society of  
Toxicology

[www.toxicology.org](http://www.toxicology.org)