

**PS 1147** Direct Interaction between Multiwalled Carbon Nanotubes and the Coronary Microcirculation.

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Recently our collaborative group identified multi-walled carbon nanotube (MWCNT) translocation from the lung to the heart, liver, and kidneys within 24 hours after pulmonary exposure. From this finding, we continued to examine the microvascular ramifications associated with this direct interaction. To model drug delivery rats, as well as lung migration, MWCNT were injected into the tail vein of rats (25-900 µg, suspended in 900 µL normosol). 24-hours later, coronary arterioles (<170 µm in diameter) from the left anterior descending artery distribution were isolated for reactivity assessments based on responses to transmural pressure (myogenic responsiveness), intraluminal flow (shear stress), phenylephrine (10-9-10-4 M), acetylcholine (10-9-10-4 M), A23187 (10-9-10-5 M), and spermineNONOate (10-9-10-4 M). Myogenic responsiveness was not altered after MWCNT injection. However, MWCNT injection at all concentrations robustly attenuated reactivity. The coronary microvascular dysfunction associated with MWCNT injection is significant, impacting endothelium-dependent, -independent, adrenergic, and flow-mediated dilation pathways. These alterations, in combination with previous findings, indicate that the microvascular impairments that follow MWCNT injection are more severe than those observed after ingestion or inhalation exposure. Studies are currently underway to further evaluate mechanistic differences between these routes of exposure. NIH-RO1-ES015022 and RC1-ES018274 (TRN)

**PS 1148** Phosgene-Induced Lethal Lung Edema Correlate with Persistent Stimulation of Cardiopulmonary Reflexes.

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Phosgene gas is a lower respiratory tract irritant. As such, it stimulates nociceptive vagal C-fiber related reflexes in a dose-rate and concentration x exposure duration (Cxt)-dependent manner. In rats this reflex is characterized by extended apnea time periods, bradycardia, and hypothermia. While inhalation exposures at non-lethal Cxt products show rapid reversibility of reflexively induced changes in respiratory patterns, lethal Cxt products seem to cause their prolonged stimulation after discontinued exposure to phosgene. This observation has been taken as indirect evidence that phosgene-induced lethal lung edema is likely to be caused by dysfunctional neurogenic control of cardiopulmonary and microvascular physiology. In order to verify this hypothesis, data from respiratory function measurements during and after the inhalation exposure to phosgene gas were compared with time-course measurements of respiratory and cardiac function over 20 hours post-phosgene exposure. These data were complemented by time-course analyses of nitric oxide (NOe) and carbon dioxide in exhaled breath, including time-dependent changes of extravasated protein in bronchoalveolar lavage fluid (BALF) and hemoglobin in blood. The nitric oxidase synthetase inhibitors L-NAME and L-NIL were used to further elucidate the role of NOe in this type of acute lung injury and whether its analysis can serve as an early biomarker of pulmonary injury. Collectively, the sequence and time-course of pathological events in phosgene-induced lung edema appear to suggest that over-stimulated, continued sensorimotor vagal reflexes trigger changes in cardio-pulmonary hemodynamics. In the absence of any successful intervention, this imbalance progresses eventually to a refractory, self-perpetuating and self-amplifying acute lung edema within 24 hours post-phosgene exposure. The continued excessive parasympathetic tone appears to be the major etiopathology in this type of high permeability lung edema following acute high-level exposures to phosgene gas.

**PS 1149** Role of Nrf2 Antioxidant Defense in Mitigating Cadmium-Induced Oxidative Stress in the Olfactory System of Zebrafish.

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Exposure to trace metals can disrupt olfactory function in fish leading to a loss of behaviors critical to survival. Cadmium (Cd) is an olfactory toxicant that elicits cellular oxidative stress as a mechanism of toxicity while also inducing protective cellular antioxidant genes via activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway. However, the molecular mechanisms of Cd-induced olfactory injury have not been characterized. In the present study, we investigated the role of

the Nrf2-mediated antioxidant defense pathway in protecting against Cd-induced olfactory injury in zebrafish. A dose-dependent induction of Nrf2-regulated antioxidant genes associated with cellular responses to oxidative stress was observed in the olfactory system of adult zebrafish following 24 h Cd exposure. Zebrafish larvae exposed to Cd for 3 h showed increased glutathione S-transferase pi (*gst pi*), glutamate-cysteine ligase catalytic subunit (*gclc*), heme oxygenase 1 (*hmox1*) and peroxiredoxin 1 (*prdx1*) mRNA levels indicative of Nrf2 activation, and which were blocked by morpholino-mediated Nrf2 knockdown. The inhibition of antioxidant gene induction in Cd-exposed Nrf2 morphants was associated with disruption of olfactory driven behaviors, increased cell death and loss of olfactory sensory neurons (OSNs). Nrf2 morphants also exhibited a downregulation of OSN-specific genes after Cd exposure. Pre-incubation of embryos with sulforaphane (SFN) partially protected against Cd-induced olfactory tissue damage. Collectively, our results indicate that oxidative stress is an important mechanism of Cd-mediated injury in the zebrafish olfactory system. Moreover, the Nrf2 pathway plays a protective role against cellular oxidative damage and is important in maintaining zebrafish olfactory function. This work was supported by the University of Washington Superfund Research Program (NIEHS P42ES004696).

**PS 1150** Cadmium Enhances Instability of Lysyl Oxidase Messenger RNA.

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Lysyl oxidase (LO), a copper-dependent enzyme, catalyzes crosslinking of collagen and elastin essential for tissue and organ morphogenesis and repair. Our previous studies have shown the critical role of downregulation of LO in cadmium (Cd)-induced emphysema pathogenesis *in vitro* and *in vivo*. The present studies further investigate Cd effects on posttranscriptional modification of the LO gene in rat fetal lung fibroblasts (RFL6). Treatment of cells with Cd (1-5 µM) inhibited levels of LO steady-state mRNAs in a dose-dependent manner. RFL6 cells displayed a relatively stable LO mRNA stability as assessed by the actinomycin D (an inhibitor of mRNA synthesis, 5 µg/ml) chase assay with the  $t_{1/2} = 24$  h. In contrast, in cells treated with 5 µM Cd plus actinomycin D, the  $t_{1/2}$  for LO mRNA decay was reduced to 0.75-h (45 min). Thus, Cd facilitates the LO mRNA decay. Rat LO mRNA contains two AU-rich elements (ARE, AUUUA), the stability determinant, at 174/178 and 200/204 downstream of the translation stop codon in the 3'-UTR. We cloned the entire LO 3'-UTR (1/229) and the ARE fragment (152/229) into the pGL3-Promoter vector after the coding region of the SV40 promoter-driven luciferase gene. The LO 3'-UTR or the ARE fragment strongly stabilized the reporter mRNAs manifested by increased luciferase activities in transfected cells. Mutation of two AREs abolished the enhancement of reporter mRNA stabilities by the LO 3'-UTR or the ARE fragment. Notably, luciferase activities driven by the LO 3'-UTR or ARE in recombinant constructs were significantly inhibited in cells treated with 5 µM Cd, indicating Cd enhancement of the decay of LO 3'-UTR or ARE-driven reporter mRNAs. HuR, a major ARE binding protein, enhances the mRNA stability. RNA immunoprecipitation (RIP) assay indicated that Cd effectively blocked the binding of HuR into the LO mRNA AREs. Thus, Cd targeting the HuR may be a critical mechanism for LO mRNA instability by this metal ion (supported by the grant of NIEHS 011340).

**PS 1151** Involvement of Inhibition of UBE2D Family Gene Expressions on Cadmium-Induced p53 Dependent Apoptosis in Human Proximal Tubular Cells.

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Cadmium (Cd), known to be a causative agent of *Itai-itai* disease, produces severe toxic effects in the kidney, liver, lung and bone. Particularly, chronic exposure to Cd causes renal dysfunction. Recently, we have found that overaccumulation of p53 may relate to Cd-induced apoptosis and may be due to the suppression of p53 degradation through the inhibition of gene expressions of Ube2d family in rat proximal tubular cells (NRK-52E cells). In this study, we examined the effects of Cd on the expressions of UBE2D family, accumulation of p53 and apoptosis in human proximal tubular cells (HK-2 cells). TUNEL positive cells, indicators of apoptosis, were increased by treatment with 20 µM Cd for 18 h. Moreover, using real-time RT-PCR, we demonstrated that Cd caused significant decrease of UBE2D mRNA levels from 6-h treatment, and UBE2D4 mRNA levels from 12-h treatment. Western blot analysis showed that Cd drastically increased p53 protein levels in HK-2 cells, even though the mRNA levels of p53 were decreased by Cd. Additionally, knockdown of UBE2D family genes by siRNA increased cellular protein levels of p53. These results indicate that Cd induces apoptosis through p53 accumulated by suppressions of UBE2D family gene expressions, in human proximal tubular cells as well as in rat proximal tubular cells.

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