

## **Lung toxicity, deposition, and clearance of thermal spray coating particles with different metal profiles after inhalation in rats\_dataset**

### **Introductory Information**

Thermal spray coating is a process in which molten metal is sprayed onto a surface. Little is known about the health effects associated with these aerosols. Sprague-Dawley rats were exposed to aerosols ( $25 \text{ mg/m}^3 \times 4 \text{ hr/d} \times 4 \text{ d}$ ) generated during thermal spray coating using different consumables [i.e., stainless-steel wire (PMET731), Ni-based wire (PMET885), Zn-based wire (PMET540)]. Control animals received air. Bronchoalveolar lavage was performed at 4 and 30 d post-exposure to assess lung toxicity. The particles were chain-like agglomerates and similar in size (310–378 nm). Inhalation of PMET885 aerosol caused a significant increase in lung injury and inflammation at both time points. Inhalation of PMET540 aerosol caused a slight but significant increase in lung toxicity at 4 but not 30 d. Exposure to PMET731 aerosol had no effect on lung toxicity. Overall, the lung responses were in the order: PMET885>>PMET540>PMT731. Following a shorter exposure ( $25 \text{ mg/m}^3 \times 4 \text{ h/d} \times 1 \text{ d}$ ), lung burdens of metals from the different aerosols were determined by ICP-AES at 0, 1, 4 and 30 d post-exposure. Zn was cleared from the lungs at the fastest rate with complete clearance by 4 d post-exposure. Ni, Cr, and Mn had similar rates of clearance as nearly half of the deposited metal was cleared by 4 d. A small but significant percentage of each of these metals persisted in the lungs at 30 d. The pulmonary clearance of Fe was difficult to assess because of inherently high levels of Fe in control lungs.

### **Methods Collection**

#### **Thermal Spray Coating Aerosol Generation and Exposure System**

-A computer-controlled thermal spray coating generator and inhalation exposure system was constructed to perform animal studies to mimic workplace exposures in a laboratory.

-The different thermal spray coating aerosols were generated in a closed spray booth and transported to an animal exposure chamber where they were collected and characterized.

-The thermal spray coating exposure system was divided into two separated areas: (A) an enclosed room where the spray coating occurred that contained a compressed air tank, thermal spray machine with wire holder and feeder unit, the rotary and reciprocating system that holds and rotates the stainless-steel pipe to be spray coated in an up and down manner, and a spray coating booth that housed the torch gun and rotary sample holder system; (B) the animal exposure chamber with different particle characterization and chamber condition devices and air flow controllers.

The mass concentration in the chamber was monitored by a real-time aerosol monitor (DataRAM). The sensors and measurement devices were managed and controlled through a custom computer software program written in LabVIEW.

-Additional ports were located on the top of the chamber and used to measure chamber pressure and to collect additional particle samples for size distribution, chemical composition, and electron microscopy analyses.

#### Thermal Spray Coating Aerosol Characterization

-*Particle size and morphology.* The size distribution of the different thermal spray coating aerosols inside the exposure chamber was determined using a Micro-Orifice Uniform Deposit Impactor. To assess particle morphology, the aerosolized particles from thermal spray coating were collected using 47-mm cassettes loaded with polycarbonate filters. The filters loaded with the collected particles were mounted onto aluminum stubs using double-stick carbon tape and viewed using a Hitachi S4800 field emission scanning electron microscope.

-*Metal composition.* Particle samples were collected inside the exposure chamber onto 5 mm pore size polyvinyl chloride membrane filters in 37-mm cassettes during thermal spray coating using the different consumable wires. The collected samples were analyzed for metal components using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) according to NIOSH Method 7303 modified for hot block/HCl/HNO<sub>3</sub> digestion.

-*Electron Paramagnetic Resonance.* To detect and measure short-lived free radical intermediates, electron paramagnetic resonance (EPR) spin-trapping was used. Final concentrations were 100 mM of the spin-trap DMPO (5,5'-dimethylpyrroline N-oxide), 5 mg/ml the thermal spray coating particle samples or potassium dichromate [2 mM; Cr (VI) positive control], and 1 mM H<sub>2</sub>O<sub>2</sub> suspended in PBS and mixed in the order listed. All reagents were mixed in test tubes for 3 min at room temperature, filtered through a Titan3 nylon 0.45 mm filter to halt the reaction and remove any metal particles. The sample was then transferred to a quartz flat cell for EPR measurement in a Bruker EMX spectrometer.

#### Animals

-*Animals.* Male Sprague-Dawley rats were used for the project and were free of viral pathogens, parasites, mycoplasmas, *Helicobacter*, and CAR bacillus and were provided HEPA-filtered air, irradiated Teklad 2918 diet, and tap water *ad libitum*.

#### Lung Toxicity Study

*-Inhalation Exposure.* The rats were exposed to the aerosols (target concentration: 25 mg/m<sup>3</sup> x 4 hr/d x 4 d) generated from electric arc wire- thermal spray coating using (1) a stainless-steel consumable wire (PMET731), (2) a Ni-based consumable wire (PMET885), and (3) a Zn-based consumable wire (PMET540). Control animals were exposed to filtered air. Different sets of exposed animals were harvested at 4 and 30 d after the last exposure. Animal body weights were measured during the exposure period and throughout the 30 d post-exposure period.

*-Bronchoalveolar Lavage.* At 4 and 30 d after the final inhalation exposure, bronchoalveolar lavage (BAL) was performed to assess lung injury and inflammation. The cell pellets from all washes for each rat were combined, re-suspended in 1 ml of PBS buffer, counted, and differentiated.

*-Assessment of Lung Injury and Inflammation.* Lactate dehydrogenase (LDH) was measured in the first fraction of the cell-free supernatant recovered from the BALF as a general marker for lung toxicity. For the determination of lung inflammation, total cell numbers recovered by BAL from the right lung were determined using a Coulter Multisizer II and AccuComp software. Cell suspensions (5x10<sup>4</sup> cells) were spun using a Cytospin 3 centrifuge. Cells (200/rat) were identified after labeling with Leukostat stain as monocytes/alveolar macrophages (AM), polymorphonuclear leukocytes (PMN), and eosinophils (EOS).

#### Lung Deposition and Clearance Study

*-Inhalation Exposure.* The rats were exposed to the respirable portion of aerosols (target concentration: 25 mg/m<sup>3</sup> x 4 hr/d x 1 d) generated from electric arc wire- thermal spray coating using (1) a stainless-steel consumable wire (PMET731; settings of 60 psi, 30 V, 200 A), (2) a Ni-based consumable wire (PMET885; settings of 60 psi, 30 V, 250 A), and (3) a Zn-based consumable wire (PMET540; settings of 60 psi, 23 V, 200 A). Control animals were exposed to filtered air. Rats (n = 6/group) were harvested at days 0 (immediately following the exposure), 1, 4, and 30 d after the exposure. Due to space limitations, two exposures were performed for each of the three consumables.

*-Lung Deposition and Clearance of Metals.* To assess the pulmonary clearance of deposited thermal spray coating particles, the metal content present in the lungs was measured on days 0, 1, 4 and 30 after the single 4-hr exposure. The concentration of specific metals deposited in the lungs at each time point was determined by ICP-AES according to NIOSH method 7300 (NIOSH, 1994).

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