

## **Development of a thermal spray coating aerosol generator and inhalation exposure system**

**Dataset Number:** \_\_\_\_\_

### **Introductory Information**

Thermal spray coating involves spraying a product that is melted by extremely high temperatures and then applied under pressure under pressure onto a surface. Large amounts of a complex metal aerosol (e.g., Fe, Cr, Ni, Zn) are formed during the process, presenting a potentially serious risk to the operator. Information about the health effects associated with exposure to these aerosols is lacking. Even less is known about the chemical and physical properties of these aerosols. The goal was to develop and test an automated thermal spray coating aerosol generator and inhalation exposure system that would simulate workplace exposures. An electric arc wire- thermal spray coating aerosol generator and exposure system was designed and separated into two areas: (A) an enclosed room where the spray coating occurs; (B) an exposure chamber with different measurement devices and controllers. The physicochemical properties of aerosols generated during electric arc wire- thermal spray coating using five different consumable wires were examined. The generated particles regardless of composition were poorly soluble, complex metal oxides and mostly arranged as chain-like agglomerates and similar in size distribution as determined by MOUDI and ELPI. To allow for continuous, sequential spray coating during a 4-hr exposure period, a motor rotated the metal pipe to be coated in a circular and up-and-down direction. In a pilot animal study, male Sprague-Dawley rats were exposed to aerosols ( $25 \text{ mg/m}^3 \times 4 \text{ hr/d} \times 9 \text{ d}$ ) generated from electric arc wire- thermal spray coating using a stainless-steel PMET720 consumable wire. The targeted exposure chamber concentration was achieved and maintained during a 4-hr period. At 1 d after exposure, lung injury and inflammation were significantly elevated in the group exposed to the thermal spray coating aerosol compared to the air control group. A thermal spray coating inhalation system was designed and constructed that will generate continuous metal spray coating aerosols at a targeted concentration for extended periods of time without interruption for future animal exposure studies.

### **Methods Collection**

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

-An automated, computer-controlled thermal spray coating particle generator and inhalation system was designed and constructed to perform animal studies to mimic workplace exposures.

-The electric arc wire- thermal spray coating aerosol generation and exposure system is separated into two areas: (A) an enclosed room where the spray coating occurs; (B) the exposure chamber with different measurement devices and 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.

-Ports installed inside the animal chamber were used for gravimetric sampling pumps to also determine particle concentration using cassettes with 47-mm filters. This filter data was used every exposure run to validate and calibrate the DataRAM. The target exposure chamber mass concentration could be selected in the software and was typically set to 25 to 30 mg/m<sup>3</sup> for all experiments.

-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 for electron microscopy analysis. The air pressure and temperature and relative humidity inside the chamber were continually measured during the exposure period.

-The physicochemical properties of aerosols generated during electric arc wire- thermal spray coating using five different and commonly used consumable wires were examined, including two stainless-steel wires (PMET720 and PMET731), two Ni-based wires (PMET876 and PMET885), and one Zn-based wire (PMET540). In addition, a study of PMET720 using two different compressed air pressures (50 and 60 psi) was performed to assess whether it affected the physical and/or chemical characteristics of the generated particles.

#### Thermal Spray Coating Aerosol Characterization

-Particle size and morphology. The size distribution of the different thermal spray coating aerosols inside the exposure chamber were determined by two methods: (1) Micro-Orifice Uniform Deposit Impactor (MOUDI); (2) electrical low-pressure impactor (ELPI). To assess particle morphology, the different thermal spray coating particles were collected onto 47-mm filters and were viewed using a Hitachi S4800 field emission scanning electron. Elemental profiles were determined by energy dispersive X-ray spectroscopy analysis to map specific metal components of the particle samples.

-Metal Composition. Particle samples were collected inside the exposure chamber onto 5 µm filters in 37-mm cassettes during thermal spray coating using the different consumable wires. The particle samples were digested, and the metals analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

## Animals and Exposure Pilot Study

-Male Sprague-Dawley rats (250 - 300 g) were used and free of viral pathogens, parasites, mycoplasmas, *Helicobacter*, and *CAR Bacillus*. The rats were acclimated for one week after arrival and provided food and water *ad libitum*.

-The rats were exposed to aerosols ( $25 \text{ mg/m}^3 \times 4 \text{ hr/d} \times 9 \text{ d}$ ) generated from electric arc wire-thermal spray coating using a stainless-steel consumable wire (PMET720) at settings of 200 A, 60 psi, and 30 V. Control animals were exposed to filtered air. At 1 d after the final exposure, bronchoalveolar lavage (BAL) was performed to assess lung injury and inflammation.

-Animals were euthanized with an overdose of a pentobarbital-based euthanasia solution ( $>100 \text{ mg/kg}$  body weight, IP; Fatal-Plus Solution, Vortech Pharmaceutical, Inc., Dearborn, MI, USA) and then exsanguinated by severing the abdominal aorta.

-The right lung was first lavaged with a 1 ml/100 g body weight aliquot of calcium- and magnesium-free phosphate-buffered saline (PBS), pH 7.4. The first fraction of recovered bronchoalveolar lavage fluid (BALF) was centrifuged at  $500 \times g$  for 10 minutes, and the resultant cell-free supernatant was saved for lung cell damage analysis. The right lung was further lavaged with 6 ml aliquots of PBS until 30 ml were collected. These samples also were centrifuged for 10 minutes at  $500 \times g$  and the cell-free BALF discarded. The cell pellets from all washes for each rat were combined, washed, re-suspended in 1 ml of PBS buffer, counted, and differentiated.

-For the determination of lung inflammation, total cell numbers recovered by BAL were determined using a Coulter Multisizer II and AccuComp software. Cell suspensions ( $5 \times 10^4$  cells) were spun using a Cytospin 3 centrifuge (Shandon Life Sciences International, Cheshire, England) for 5 minutes at 800 rpm onto a slide. Cells (200/rat) were identified after labeling with Leukostat stain (Fisher Scientific, Pittsburgh, PA, USA) as alveolar macrophages (AMs) and neutrophils (PMNs).

-Lactate dehydrogenase (LDH) was measured in the first fraction of the cell-free supernatant recovered from the BALF as a general marker for lung cell injury. LDH activity was determined by measuring the oxidation of lactate to pyruvate coupled with the formation of NADH at 340 nm. Measurements were taken with a COBAS MIRA auto-analyzer.

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