

### Overview of the project

*Introduction:* Thermal spray, in general, is a process that involves forcing a melted substance, such as metal or ceramic in the form of wire or powder, onto the surface of a targeted object to enhance its desired surface properties. In this paper, the melted substance is metal wire generated by an electric arc and forcibly coated on a rotary iron substrate using compressed air. This thermal process is referred to as double-wire arc thermal spray. The particles generated through these methods fall within the nanometer to micrometer agglomerate size range. There is concern regarding potential human health outcomes as these particles exhibit a similarity in particle morphology to welding fumes. Thermal spray wires with Zn (PMET540), Fe and Cr (PMET731), and Ni (PMET885) as primary metal compositions were used to generate particulate via an electric arc wire thermal spray generator for exposure to human bronchial cells (BEAS-2B) to examine comparative toxicity ranging from 0-200 µg/mL. Resulting cellular viability was assessed through live cell counts, and percent cytotoxicity was measured as a function of LDH release. Oxidative stress, genotoxicity, and alteration in Total Antioxidant Capacity were evaluated through DNA damage (COMET analysis) and antioxidant concentration at 0, 3.125, 25, and 100 µg/mL. Protein markers for Endothelin-1 (ET-1), Interleukin-6 (IL-6), and Interleukin-8 (IL-8) were also assessed to determine inflammation and endothelial alteration.

#### *Methods collection:*

- Human bronchial (BEAS-2B) grown in submerged culture until confluent in a flask; seeded into plate wells at known, uniform concentrations prior to exposure.
- Thermal spray particles suspended in solution and sonicated to remove aggregates. Stock suspension was immediately serially diluted to generate dosages for exposure. Suspended dose-level concentrations applied directly to submerged cell cultures.
- Living cell counts were used as a proxy for percent viability of cultures. Counts were conducted using standard trypsinization, Trypan Blue, and a Countess II cell counter.
- Lactate Dehydrogenase (LDH) release was used to estimate cytotoxicity in response to thermal spray exposure.
- DNA Breakage was evaluated through the Trevigen COMET analysis. Cells were suspended in a porous agar matrix, size separated via electrophoresis, and stained to generate quantitative data surrounding DNA damage post-exposure.
- The Total Antioxidant Capacity kit was employed to quantify cellular production of non-enzymatic antioxidants via colorimetric shift generated by the reduction of  $\text{Cu}^{2+}$ .
- ELISA Assays were conducted for Endothelin-1, Interleukin-6, and Interleukin-8 to assess protein concentration of three secreted protein markers associated with vasoconstriction, inflammation, and immune response. Culture fluid was incubated with primary and then secondary antibodies, in sequence, before addition of colorimetric probes to quantitate bound protein concentrations.

*Citations:* Burns, E. S., Harner, R. E., Kodali, V., Afshari, A. A., Antonini, J. M., & Leonard, S. S. (2024).

Comparative in vitro toxicity of compositionally distinct thermal spray particulates in human bronchial cells. *Toxicology Reports*, 101851.

*Acknowledgements:* When a publication makes use of this data set, acknowledgement of the development of the data set should be attributed to National Institute for Occupational Safety and Health (NIOSH), Health Effects Laboratory Division (HELD). Ellie S. Burns (txq5@cdc.gov), Rylee E. Harner (reh00011@mix.wvu.edu), Vamsi Kodali (ywu0@cdc.gov), Aliakbar A. Afshari (aca8@cdc.gov), James M. Antonini (jga6@cdc.gov), Stephen S. Leonard (sel5@cdc.gov)

*Project support:* National Occupational Research Agenda Pilot Project 9390MQB

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