## Lung Toxicity Profile of Inhaled Copper-Nickel Welding Fume in A/J Mice

### Introductory Information

The process of stainless steel welding creates fumes rich in carcinogenic metals such as chromium (Cr). Welding consumables devoid of Cr are being produced in attempt to limit worker exposures to potentially toxic and carcinogenic metals. The study objective was to characterize a copper-nickel (Cu-Ni) fume generated using gas metal arc welding (GMAW) and determine the pulmonary deposition and toxicity of the fume in mice exposed by inhalation. Male A/J mice (6 – 8 weeks of age) were exposed to air or Cu-Ni welding fumes for 2 (low deposition) or 4 (high deposition) hours/day for 10 days. Mice were sacrificed and bronchoalveolar lavage (BAL), macrophage function, and histopathological analysis were performed at various timepoints post-exposure to evaluate resolution. Characterization of the fume indicated that most of the particles were between 0.1 and 1 µm in diameter, with a mass median aerodynamic diameter of 0.43 µm. Metal content of the fume was primarily Cu (~76%) and Ni (~12%). After exposure, BAL macrophages had a reduced ability to phagocytose *E. coli* at 1 and 7 days and lung cytotoxicity was evident and significant (>12-19% fold change). Loss of body weight was also significant at these two early timepoints. Lung inflammation, the predominant lesion identified by histopathology, was observed as a subacute response early that progressively resolved by 28 days with only macrophage aggregates remaining late (84 days). Future studies are planned to investigate the tumorigenic potential of Cu-Ni fume in A/J mice.

### Methods Collection

*Animals*

- Male A/J mice aged 4-6 weeks were house in a specific pathogen-free, environmentally controlled facility.

*Welding Fume Inhalation Exposure System*

- The automated robotic welder continuously generated welding fumes by welding beads onto ¼ inch thick plates of mild steel.
- The resulting fume was carried into a whole-body exposure chamber through a flexible tube by maintaining the chamber at a negative pressure. Particle concentrations and gas generation within the exposure chamber were continuously monitored.
- Welding fume characterization was conducted on filters present in the exposure chamber that collected particles that were generated.

*Whole Lung Metal Analysis*

- Weight-matched A/J mice were exposed by whole-body inhalation in individual cages to Cu-Ni welding aerosols for 4 hours or filtered air.
- Immediately following exposure, mice were euthanized. Whole lungs were excised, trimmed, and lyophilized.
- The freeze-dried tissue was weighed then acid digested.
- ICP-AES was used to determine the amount of Al, Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Ni, P, Pb, Sr, Ti, V, Zn and Zr present in the lung.

*Assessment of Lung Toxicity*

- Mice were exposed by whole-body inhalation to Cu-Ni welding fume aerosols or filtered air for 2 [low deposition (LD)] or 4 [high deposition (HD)] hours/day for 10 days.
- Before the start of the exposure mice were weight-matched then weighed biweekly and again at the terminal sacrifice of 1,7,28, and 84 days after the 10 day exposure.
- Mice were euthanized and bronchioalveolar lavage (BAL) fluid was collected through a cannula placed in the trachea. The thorax was massaged as phosphate buffered saline (PBS) was instilled into the lungs. After 10 seconds, the BAL fluid was withdrawn and collected.
- This process was then repeated 3 times and this second fraction was collected separately.
- The first fraction of BAL fluid was used to measure LDH activity
- Cells of both fractions were combined for cell counts and differential staining. Total cell numbers were determined using a hemocytometer.
- For cell differentials, cells were plated onto glass slides, stained, and coverslipped. A minimum of 300 cells/slide, consisting of macrophages, lymphocytes, and polymorphonuclear leukocytes were identified.

*Macrophage Functional Assay*

- Lung macrophages harvested by BAL at 1, 7, and 28 days post-exposure were challenged with Escherichia coli (E. coli) GFP for 2 hours. The uptake of E.coli by macrophages was quantified by flow cytometry.

**Citations –** PC Zeidler-Erdely, A Erdely, V Kodali, RN Andrews, JM Antonini, T Trainor, R Salmen, LA Battelli, L Grose, Kashon M, S Service, W McKinney, S Stone, LM Falcone. Lung Toxicity Profile of Inhaled Copper-Nickel Welding Fume in A/J Mice. Toxicology. Inhal Toxicol. 2022 Jun 20;1-12. doi: 10.1080/08958378.2022.2089783

**Acknowledgments**

Patti Zeidler-Erdely, paz9@cdc.gov

Aaron Erdely, efi4@cdc.gov

Vamsi Kodali, ywu0@cdc.gov

Ronnee Andrews, ria0@cdc.gov

James Antonini, jga6@cdc.gov

Taylor Trainor, taylordearmitt@gmail.com

Rebecca Salmen, zvj4@cdc.gov

Lori Battelli, lob0@cdc.gov

Lindsay Grose, pzq3@cdc.gov

Michael Kashon, mqk1@cdc.gov

Samantha Service, pp3@cdc.gov

Walter McKinney, wdm9@cdc.gov

Samuel Stone, zhf9@cdc.gov

Lauryn Falcone, laurynmfalcone1@gmail.com

**Contact**

For further information contact:

Pathology and Physiology Research Branch (PPRB), Health Effects Laboratory Division (HELD), National Institute for Occupational Safety and Health (NIOSH), Morgantown, WV

304-285-5831