ABSTRACT FINAL ID: 3589 Poster Board: P281
TITLE: Development of an *In Vitro* Inhalation Toxicity Test for Improved Protection of Human Health

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: G. R. Jackson, A. Hunter, J. DeBay, P. Hayden. *MatTek Corporation, Ashland, MA.*
KEYWORDS: Inhalation Toxicology; *In Vitro* and Alternatives; Toxicity; Acute

ABSTRACT BODY: Knowledge of acute inhalation toxicity and irritation potential is important for establishing safe handling, packaging, labeling, transport and emergency response procedures for chemicals. The US EPA High Production Volume Chemical Challenge, and the EU REACH programs have further increased the need for inhalation toxicity information. A UN treaty endorsed by the US, EU and others outlines a "Globally Harmonized System" (GHS) of Classification and Labeling of Chemicals. The GHS specifies 5 inhalation toxicity categories. The EPA has established a separate system that uses 4 toxicity categories. Acute inhalation toxicity tests currently accepted within the GHS and EPA systems involve *in vivo* 4 hr rat inhalation LC50 tests (OECD TG 403/436). In the current work, a newly developed *in vitro* toxicity test was evaluated in comparison to the established *in vivo* tests. The *in vitro* test exposes an organotypic human airway tissue model to test chemicals for 3 hrs, followed by measurement of tissue viability (IC75). 64 chemicals covering a broad range of toxicity classes, chemical structures and physical properties were evaluated. Results show that the *in vivo* and *in vitro* tests had 100% concordance for identifying highly toxic chemicals (GHS Cat 1-2 and EPA CAT I-II). However, the *in vivo* tests had only 29.0% (EPA system) or 61.4% (GHS system) sensitivity for identifying less toxic respiratory irritants. Numerous human respiratory irritants including acids, bases, aldehydes, amines and others were not classified as respiratory toxins/irritants by the *in vivo* tests. The *in vitro* airway model was very good (sensitivity of 81.1 - 82.4%) for distinguishing respiratory toxins and irritants (corresponding to GHS 1-3, EPA, I-III) from non-toxins, non-irritants (corresponding to GHS 4-5, EPA IV). Overall accuracy of the *in vitro* test was 81.2 - 84.1%. There were no false negative GHS Cat 1-2 or EPA Cat I-II predictions using the *in vitro* test. These data suggest that tests based on lethality in animals, while good for predicting highly toxic chemicals, produce a high percentage of false negative predictions for moderately/slightly toxic or irritating chemicals. The *in vitro* test using an organotypic human airway model was equal to current animal tests for predicting highly toxic inhaled chemicals, and better than animal tests for predicting moderately/slightly toxic respiratory irritants. The new *in vitro* testing approach should provide improved protection of human health compared to the current animal tests.

ABSTRACT FINAL ID: 3590 Poster Board: P282
TITLE: Welding Fume-Induced Generation of Reactive Oxygen Species and Activation of Inflammatory Signaling Pathways in RAW 264.7 Mouse Macrophages

AUTHORS (FIRST INITIAL, LAST NAME) & INSTITUTIONS: J.M. Antonini, V. Kodali, L.M. Bishop, T. Eye, T.G. Meighan, A. Erdely, M. Shoeb. *NIOSH, Morgantown, WV.*
KEYWORDS: Particulates; Inhalation Toxicology; Inflammation; Metals; Welding Fume

ABSTRACT BODY: Welding fumes are a complex mixture of several toxic metals (e.g., Cr, Mn, Fe, Ni). Epidemiology indicates that welders have an increased risk for lung disease, including bronchitis, airway infections, and cancer. Animal toxicology studies show that specific welding fumes cause significant lung injury and inflammation, depending on the metal composition of the fume. The goal was to examine the potential mechanisms by which welding fumes with different metal compositions may generate reactive oxygen species (ROS) and activate inflammatory signaling pathways in an *in vitro* model. RAW 264.7 mouse macrophages were incubated with 0, 3.125, 6.25, 12.5, 25 and 50 µg/mL of either manual metal arc-stainless steel (MMA-SS) or gas metal arc-mild steel (GMA-MS) welding fumes for 24 hr. Cytotoxicity, ROS generation, and activation of different inflammatory markers were assessed. Metal composition and solubility of the fumes were different: MMA-SS (41% Fe, 29% Cr, 17% Mn) was highly water-soluble, whereas GMA-MS (85% Fe, 14% Mn) was water-insoluble. At 24 hr, MMA-SS significantly elevated ROS generation and dose-dependently increased cytotoxicity in the RAW 264.7 cells compared to GMA-MS and saline control. Welding fume-induced ROS generation led to production of the toxic lipid aldehyde, 4-hydroxynonenal (HNE). Both welding fumes induced the activation of the mitogen activated protein kinases (MAPK), such as extracellular signal-regulated kinases 1 and 2 (ERK1/2), leading to the increased expression of COX-2 in the RAW 264.7 cells. Also, welding fumes increased protein expression of Nrf2 and HO-1, activating the Nrf2-Keap-HO-1 pathway. In all cases, a significantly greater activation of the different inflammatory markers was observed for MMA-SS compared to the GMA-MS and saline control. These results suggest that the cytotoxicity of RAW 264.7 cells caused by MMA-SS is due to the presence of soluble and cytotoxic metals (Cr, Ni) that are absent in the GMA-MS fume.

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of and Increasing the Impact of Toxicology

2016 Annual Meeting Abstract Supplement

Late-Breaking Abstract Submissions

All Late-Breaking Abstracts are presented on Thursday, March 17, from 9:30 am–12:45 pm.

These abstracts are available via the Mobile Event App, Online Planner, and a downloadable PDF from the SOT website.

55th Annual Meeting and ToxExpo™

March 13–17, 2016

New Orleans, Louisiana

SOT