1313 Toxicological Effects of Inhaled Fracking Sand Dust on Reactivity and Neurogenic Responses of Isolated Rat Trachea

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Hydraulic fracturing (fracking) entails pumping a fluid and sand (silica) mixture through drilled wells to cause the fracturing of shale deep underground, resulting in the release of natural gas. During this process, workers at fracking sites are exposed to large amounts of respirable sand dust as it is prepared for pumping into the well. Inhalation exposure to high levels of crystalline silica is known to produce silicosis. To investigate the toxicological effects of inhaled fracking dust, rats were exposed to fracking sand dust (collected at a fracking site) at 10 mg/m3 or 30 mg/m3 for 6 hours per day for 4 consecutive days. Tracheas were isolated from euthanized animals for *in vitro* preparations. In the isolated, perfused trachea at day 7 post-exposure to 10 mg/m3 the reactivity to methacholine (MCh) applied to the extraluminal surface of the trachea was increased as compared to air-breathing control animals. Contractile responses to intraluminally-applied MCh were unaffected by dust inhalation at any of the post-exposure time points examined. In tracheal strips, electrical field stimulation evoked contractions that were not affected by dust inhalation of 10 mg/m3 or 30 mg/m3 at any of the post-exposure time points. These findings suggest that at 10 mg/m3, reactivity of airway smooth muscle to methacholine was increased but effector nerve function was not altered at either 10 or 30 mg/m3. No changes to the integrity of the epithelial cell lining of the trachea were observed.

1314a Role of Osteopontin & Proteinase 3 in BAL & Blood in Phosgene-Induced Lung Edema

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The intervention principles of phosgene-induced pulmonary edema are controversially discussed and commonly countermeasures focus on anti-inflammatory principles. One of the key mechanism of phosgene is to deplete glutathione (GSH) in the lining fluids of the lung and the function of alveolar macrophages (AM) is increasingly compromised due to the limited availability of GSH. Recent evidence suggests that any reduction of the redistribution of vascular fluid from the systemic circulation into the lung prevents edema occurrence and progression if administered early enough postexposure. This study examined rats exposed to 1000 mg/m^3 x min phosgene gas. Air exposed rats served as control. All animals were examined on the climax of edema occurrence 1-day post-exposure. Leukocytes (neutrophilic granulocytes, PMN), osteopontin (OPN) and PMN-specific proteinase (PR3) were analyzed in blood. Red blood cells (RBC) counts and hematocrit were used to estimate any degree of hemoconcentration. These endpoints were compared with PMN-counts, OPN- and PR3-protein in bronchoalveolar lavage (BAL)-fluid and BAL-cells. Total protein and collagen in BAL-fluid served as endpoints mirroring the magnitude of edema formation. Hemoconcentration coincided with significantly decreased PMN counts and PR3 in blood. This was contrasted by increased BAL-PMN counts and PR3 in BAL-cells. AM were decreased by 50% and 1/3 of them had a foamy appearance. OPN in BAL-cells was minimal relative to BAL-fluid. The most salient increase of OPN occurred in plasma. In summary, the extravasation of collagen into the alveolus paralleled that of total protein. The changes of PR3 appear to mirror the counts of PMN in the respective compartment. Its moderate increase supports the conclusion that PMN migrate into the lung concomitant with extravasated proteins in the absence of any appreciable activation. AM appear to be particularly susceptible to an almost lethal exposure dose of phosgene. More research seems to be warranted on early countermeasures focusing on maintaining the functional integrity of the AM to minimize the generation of metabolites affecting cardiopulmonary function rather than modulating any PMN-related inflammatory responses.

1314 Instillation of Indium-Tin Oxide Production Facility Particles in Rats Induces Pulmonary Toxicity

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Indium-tin oxide (ITO) is a mixture of metal oxides and is used to make transparent conductive coatings for touch-screen and liquid crystal display electronics. As the demand for consumer electronics continues to rise, concern over occupational exposures to potentially toxic particles generated during ITO production has increased. Epidemiologic studies have shown an association between workers exposed to ITO and development of pulmonary alveolar proteinosis (PAP) and fibrotic interstitial lung disease. Our previous *in vitro* studies have demonstrated cytotoxicity and inflammasome activation in response to various indium-containing particles. However, which of these indium materials or specific industrial processes may be the most toxic *in vivo* remains unknown. In the current study, particle samples were collected at different production stages throughout an ITO facility and various endpoints were evaluated over a time course following rat pulmonary exposures to these particles. Indium oxide (In2O3) is a starting material, sintered ITO (SITO) is generated during the grinding of the final ITO product, and ventilation dust (VD) is present during reclamation of indium from left-over materials (and therefore, contains SITO). Rats were exposed via a single intratracheal instillation dose of 0 mg (PBS vehicle control), 0.5 mg (VD only), 1 mg, or 5 mg per rat (n = 6-8/group). Each particle type induced pulmonary inflammation and damage in rats (evaluated at 1, 7, and 90 days post-instillation), but SITO and VD caused the most damage when responses at the same dose were compared on days 7 and 90. SITO and VD also led to significantly higher plasma indium levels than In2O3. Downstream pathological changes such as PAP and fibrosis were observed in response to all three particles 90 days post-treatment, with a trend towards greatest severity in animals exposed to VD when comparing animals that received the same dose. These findings may inform workplace exposure reduction efforts and provide a better understanding of the pathogenesis of this emerging occupational health issue.

1315 A Comparative Study of Essential Oils and Their Chemotypes on A549 Lung Cancer Cells

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Purpose: Essential oils (EOs) are concentrated from plants and carry a distinctive "essence" that is not limited to perfumery. Essential oils have been used for healing properties and may provide an adjuvant method for treating diseases such as lung cancer. While some studies examine constituents of specific compounds on various cancer cells lines, current literature is limited on chemotype apoptotic effects. A chemotype is a chemically distinct entity within a plant species; Thymus vulgaris has 7 possible chemotypes, each of which will change the properties of the EO if it is the dominant component. We hypothesize that EOs (frankincense, oregano, nutmeg, myrrh) and thyme chemotypes (Thyme thujanol, Thyme linalool, Thyme thymol) will induce apoptosis in lung (A549) cancer cell lines. The objective of this study was to determine if thyme EOs containing different chemotypes have altered apoptotic activity in A549 cells. Methods: Cells were cultured and maintained using standard ATCC protocols. Cell viability was determined by Alamar Blue assays. Cells were plated in 96-well plates and serial dilutions of EOs/chemotypes were added for 12 hours with either 1:250, 1:500, 1:750, or 1:1000 dilutions of EOs and compared to control. Intracellular caspase detection was assessed at 24 hours using the same methodology. Based on results chemotypes were screened using HPLC for constituent activity. Results: Data suggests oregano and the thyme chemotypes exhibited the best cytotoxic activity against A549 cells. Viability decreased by 20% for oregano at concentration ratios of 1:500 and 1:750; Thyme linalool at 1:250; and Thyme thymol at 1:500, 1:750, and 1:1000. Caspase activation was observed in Thyme linalool, and Thyme thymol chemotypes. HPLC data confirmed that active chemotypes (Thyme linalool, and Thyme thymol) contained a distinct peak for thymol, while Thyme thujanol did not contain thymol. Data are the average of three independent experiments ± (SEM), $p < 0.05$. Conclusions: Essentials oil chemotypes containing thymol exhibited the greatest decrease in cell viability and demonstrated caspase activation. However, not all chemotypes within the same plant species contain the same constituents. When considering EO use, plant chemotypes may play an important role in determining cancer cell viability.

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. Author names which are underlined in the author block indicate the author is a member of the Society of Toxicology. For example, J. Smith.

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