1391 ROLE OF IRON IN THE GENERATION OF RADICALS BY FRESHLY FRACTURED SILICA: AUGMENTATION OF PULMONARY REACTIONS TO SILICA INHALATION

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Fracturing quartz produces radicals on the fracture planes and generates hydroxyl radicals (OH) in aqueous media. OH production is directly associated with silica-induced cell damage and phagocyte activation in vitro. This OH production in vitro is inhibited by desferol, an iron chelator. The present objective was to determine if iron increased the ability of inhaled silica to cause inflammation and lung injury. Male Fischer rats were exposed 5 hrs/day for 10 days to filtered air, 20 mg/m^3 freshly milled silica (30–40 ppm iron), or 20mg/m^3 freshly milled silica doped with iron (>500 ppm iron). High iron silica produced ≈ 70% more radicals in vitro than the low iron silica. Compared to inhalation of low iron silica, high iron silica resulted in greater neutrophil recruitment (↑900%), macrophage production of oxygen radicals measured by electron spin resonance or chemiluminescence (↑32% or 90%, respectively), nitric oxide production by macrophages (↑71%), and lipid peroxidation of lung tissue (↑38%). The results suggest that inhalation of a mixture of iron and freshly fractured silica may be more pathogenic than inhalation of silica alone.

1392 THE ROLE OF METAL-CATALYZED OXIDANTS IN LUNG INJURY AFTER PARTICLE EXPOSURE

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We tested the hypothesis that a metal initially complexed to the surface of a particle could catalyze the generation of oxidants and be associated with an inflammatory injury of the lung. Polystyrene beads (0.945 μm; Bangs Laboratories, Carmel, IN) with surface -COOH groups were treated with NaOH and then agitated in either 100 μM ferric ammonium sulfate (latex-Fe^{3+}) or sterile water (latex) for 30 minutes. *In vitro* oxidant generation was measured as the absorbance of thiobarbituric-acid-products (A_{532}) in reaction mixtures including 1.0 mM H_2O_2, 1.0 mM ascorbate, 1.0 mM deoxyribose, and particles. Incubations with 1000 μg/ml latex-Fe^{3+} were increased relative to those including either an identical mass of latex or saline (A_{532} of 1.146 ± 0.036, 0.372 ± 0.015, and 0.093 ± 0.016 respectively; mean ± standard deviation). Similarly, maximal luminol-enhanced chemiluminescence by rat alveolar macrophages (1.0 × 10^6 cells/ml) was elevated with exposures to 1000 μg/ml particles with complexed metal (3.0 ± 0.1, 0.2 ± 0.1, and 0.0 ± 0.0 V). Finally, 24 hours after intratracheal instillation into 60 day old, male Sprague Dawley rats, inflammation was significantly increased after latex-Fe^{3+} (lavage neutrophils of 37 ± 11%, 16 ± 9%, and 2 ± 3% and lavage proteins concentrations of 0.51 ± 0.05, 0.26 ± 0.05, and 0.11 ± 0.03 mg/ml). We conclude that metal initially complexed to the surface of a latex particle can be associated with increments in oxidant generation and lung injury. This abstract does not necessarily reflect EPA policy.

1393 SUSCEPTIBILITY AND DIET: THE ROLE OF VITAMIN C IN ACUTE OZONE TOXICITY IN GUINEA PIGS

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Recent evidence suggests that most Americans consume less than the optimal five servings of fruits and vegetables per day, and many have less than saturating levels of vitamin C (C) in their blood plasma. C is actively concentrated into airway lining fluids where it serves as an antioxidant to protect against oxidant pollutants. The present study investigated possible endpoints that could be used in a human study of the interaction between C and inhaled ozone (O_3). Guinea pigs were fed a C deficient chow until plasma C was ~20% of normal (2 weeks). O_3 exposure (2 hr, 1.0 and 0.4 ppm) was followed immediately by a pulmonary function test battery, and by preparation of tissues for morphological and biochemical assessment (also at 24 hr post exposure). Results showed that bronchoalveolar lavage (BAL) protein was increased by O_3 exposure and further increased by C deficiency, especially at the 0.4 ppm level. BAL neutrophil percentage and all pulmonary function measurements were altered by O_3 but not by C status. The percent denuded epithelium in the proximal alveolar region was increased from 3% in controls to 45% in O_3 (1 ppm) exposed to 62% in O_3 exposed C deficient animals. Use of labeled O_3 (oxygen-18) for exposure resulted in enhanced labeling of BAL constituents in C deficient animals, consistant with the hypothesis that C prevents O_3 from reacting with lipids and proteins of the lung lining material. *In vitro* studies confirmed that incorporation of oxygen from $^{18}O_3$ into proteins is inhibited by the presence of C in the medium. In summary, dietary C appears to alter cell injury endpoints of susceptibility to O_3 while some lung function and inflammatory indicators appear to be unaffected. (This abstract does not necessarily represent EPA policy).

1394 OZONE-INDUCED DNA-BINDING ACTIVITY OF TRANSCRIPTION FACTORS AND IL-8 PRODUCTION IN A549 CELLS

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Ozone (O_3), one of the most reactive oxidant to which humans are routinely exposed, induces inflammation in the lower airways. The airway epithelium is one of the first targets inhaled O_3 will encounter. However, its role in ozone-induced airway inflammation is not well understood. Epithelial cells could contribute to the neutrophil influx seen after exposure to O_3 by releasing interleukin-8 (IL-8), a potent neutrophil chemotactic factor. Expression of inducible genes involved in the inflammatory and immune response, such as IL-8, is controlled by transcription factors. The IL-8 gene appears to be under the control of the transcription factors NF-KB, AP-1, and NF-IL6. A human type-II-like epithelial cell line (A549) was grown to a monolayer in Costar Transwells (Costar, Cambridge, MA) and exposed *in vitro* to 0.1 ppm O_3/air. Induction of DNA-binding activity of transcription factors was assessed by mobility shift. Exposure of A549 to 0.1 ppm O_3 for 5 hours induced DNA-binding activity of NF-KB, AP-1, and NF-IL6. Additionally, the effects of O_3 on IL-8 release was measured in A549 cells 48 hours following exposure as described above. IL-8 levels in the basolateral supernatant of O_3-exposed A549 cells was significantly increased as compared to air-exposed cells. These results link ozone-induced DNA-binding activity of transcription factors and the production of IL-8 by epithelial cells, thus demonstrating a possible cellular mechanism of epithelial-mediated airway inflammation. (Sponsored by EPA R819342).

1395 PREFERENTIAL O_3 ABSORPTION SUBSTRATES: EFFECTS ON OXIDATION OF LUNG LINING LAYER LIPIDS.

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O_3 absorption occurs due to reaction within the surface lining layer (SLL) and produces oxidation of unsaturated fatty acids (UFA). However, the relationships among O_3 absorption rates, the distribution of SLL substrates, and the induction of oxidant stress remain undefined. SLL (as bronchoalveolar lavage fluid; BALF) and model systems were exposed under well-mixed, steady-state conditions (~ 0.5 ppm O_3; 25°; 20 min). Substratespecific absorption rates and UFA oxidation products (aldehydes, TBARS) were determined. **Results: a)** Ascorbate (AH_2) and egg phosphatidylcholine liposomes (EggPC) produce greater O_3 uptake than glutathione (GSH). In combined systems, AH_2 accounts for most O_3 uptake. **b)** Exposure of BALF produces O_3-specific (nonanal and heptanal) and autoxidation aldehydes (hexanal) in a dose and time-dependent manner. **c)** Addition of either BALF or AH_2 to EggPC increases O_3 uptake (AH_2 >> BALF). However, TBARS are increased by BALF but limited by AH_2. **d)** Removal of AH_2 from BALF decreases uptake but substantially increases nonanal, heptanal, and hexanal production. **Conclusions**: AH_2 and lipids are the predominant O_3 absorption substrates. AH_2 limits direct O_3 attack of UFA and quenchs O_3-induced autoxidation. However, based on the observed relationships between O_3 absorption and UFA oxidation, for a given [O_3], dose/response relationships may be complex. Due to inhomogeneities in SLL constituent profiles, increased absorption may not necessarily lead to increased oxidative events. (CIAR 90–23, HEI 91–7, NIH T32ES07254, and Meadows Foundation)

1396 EFFECTS OF ASCORBATE AND HEXAVALENT (CrVI) AND PENTAVALENT CHROMATE (Cr V) IN SEVERAL SYSTEMS, INCLUDING LUNG CELLS, AND IN VITRO

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Previous studies in our laboratories have shown that Cr(V) and reactive oxygen species (ROS) are produced in A549 cells on addition of Cr(VI), as

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Preface

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An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 351.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 375.

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