214 INFLAMMATORY EFFECTS OF QUARTZ SAMPLES AFTER INTRATRACHEAL INSTILLATION IN RATS.

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Objectives: This 28-day study compared the inflammatory potential of quartz with geologically ancient surfaces to well-characterized reference quartz DQ 12 (RQ). A differentiation of subspecies of quartz dusts is important in regard to a wide exposure and a potential carcinogenicity. Methods: A quartz sample in a naturally occurring respirable size (quartz isolate-QI) was separated centrifugally from an unprocessed bentonite dispersion, as were clay minerals (clay isolate-CI). QI contained approx. 67% quartz with the remainder being predominantly clay minerals, and CI contained <0.035% quartz. Lung effects of QI and CI were evaluated in rats at 3 and 28 days after administering a total dose of 15.2 mg/kg in 4 equal doses. To rank the effects, a positive control (RQ) and a negative control (TiO2) were included. Results: Bronchoalveolar lavage showed a severe acute inflammatory effect in the CI group at 3 days after last dosing, which recovered mostly after 28 days. QI induced moderate inflammatory effects after 3 days, which did not change significantly by 28 days post treatment. In contrast, RQ showed a high acute inflammatory response on day 3, persisting through day 28. Histopathologically pulmonary inflammation in lungs at day 28 was most pronounced in the RQ group, whereas QI and CI showed significantly lower effects. In contrast to the quartz groups, inflammation in the CI group was not persistent or progressive but was a late recovery phase of a severe acute inflammatory effect. Conclusion: Pulmonary inflammatory effects of various quartz subspecies were significantly different.

215 PULMONARY TOXICITY OF ADVANCED COMPOSITE MATERIAL COMBUSTION ATMOSPHERES IN RATS.

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Advanced composite materials (ACM) provide a lighter alternative to their metal counterparts without compromising strength. A downside to the use of ACM is their ability to burn. To investigate the toxicity of ACM combustion atmospheres F-344 rats were exposed for 1 hour in whole-body chambers to the smoke produced from the pyrolysis of 60 g of carbon-graphite/epoxy material. Control rats were exposed to filtered air. After 1, 3, and 7 days post-exposure, the animals underwent bronchoalveolar lavage. The lavaged cells were quantitated and identified morphologically. The lavage fluid was analyzed for the inflammatory cytokines Interleukin-1 beta (IL-1β), IL-6, and Tumor Necrosis Factor-alpha (TNF-α) by ELISA. The results included significant increases in cell counts, polymorphonuclear leukocytes (PMN)s, IL-1β, IL-6, and TNF-α as compared to controls. In conclusion, exposure to ACM combustion atmospheres produces time-dependent pulmonary toxicity manifested by the recruitment of inflammatory cells, release of inflammatory cytokines and the development of pulmonary inflammation.

216 ACUTE PULMONARY RESPONSE OF INDUCIBLE NITRIC OXIDE SYNTHASE KNOCKOUT *VERSUS* WILD TYPE MICE FOLLOWING ASPIRATION OF LIPOPOLYSACCHARIDE PLUS INTERFERON-γ OR QUARTZ.

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Exposure of mice to lipopolysaccharide (LPS) plus interferon-γ (IFN-γ) or to quartz increases nitric oxide (NO) production, which has been proposed to play a role in the resulting pulmonary damage and inflammation. To determine the role of NO in these acute lung reactions, the responses of inducible nitric oxide synthase knockout (iNOS KO) versus C57BL/6J wild type (WT) mice to aspiration of LPS+IFN-γ or quartz were compared. Male mice (6-8 weeks) were exposed by aspiration to LPS (1.2 mg/kg) + IFN-γ (5000 University), quartz (40 mg/kg), or saline vehicle. At 24 hours post-exposure, lungs were lavaged with 10 aliquots (1 ml each) of Ca+2 and Mg+2 free phosphate-buffered saline. The acellular fluid from the first bronchoalveolar lavage (BAL) was analyzed for total antioxidant capacity, lactate dehydrogenase (LDH) activity, albumin, tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-2 (MIP-2). The cellular fraction of the total BAL fluid was assayed for alveolar macrophage (AM) and polymorphonuclear leukocyte (PMN) counts, and AM zymosan-stimulated chemiluminescence (AM-CL). Exposure to LPS + IFN-γ decreased total antioxidant capacity, increased BAL AMs and PMNs, LDH, albumin, TNF-α and MIP-2, and enhanced AM-CL to the same extent in both iNOS KO and WT mice. Exposure to quartz decreased AM yield, increased PMNs, LDH, albumin, TNF-α and MIP-2, and enhanced AM-CL. However, iNOS KO mice exhibited less AM activation (activation status was defined as an increased AM-CL and decreased AM yield) than WT mice. These data suggest that NO may play a role in the acute pulmonary response to quartz exposure; however, evidence for a role of NO in the acute reaction to LPS+IFN-γ was not obtained.

217 INTERACTION BETWEEN PRIMARY ALVEOLAR MACROPHAGES(AM) AND PRIMARY ALVEOLAR TYPE II (TII) CELLS UNDER BASAL CONDITIONS AND AFTER LIPOPOLYSACCHARIDE (LPS) OR QUARTZ EXPOSURE.

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This study evaluated the mutual interactions between AM and TII cells under unstimulated or LPS (10 μg/ml) or quartz (100 μg/ml) exposure conditions. AM were obtained by bronchoalveolar lavage of rats, while rat TII cells were isolated by enzymatic digestion and purified by panning (~20 million cells/rat, 95% pure). AM and TII cells were co-cultured either separated by transwell inserts or in a single well to allow physical contact. After an 18 hour culture period in the absence or presence of stimulant, the medium was assayed for tumor necrosis factor-alpha (TNF-α), macrophage inflammatory protein-2 (MIP-2), interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and nitric oxide (NO). Cell viability, which was measured as lactate dehydrogenase (LDH) released from the cells into the medium, was not affected by either transwell or contact co-culture under basal or stimulated conditions. Under basal conditions, co-culture of AM and TII cells in transwells significantly potentiated the release of TNF-α, MIP-2, IL-6 and NO above the sum of the production by these cells cultured separately. Physical contact between AM and TII cells mitigated this potentiation, which was further decreased by exposure to LPS or quartz. Indeed, under stimulated conditions, physical contact actually decreased the production of some of these inflammatory products below the sum of the production by these cells cultured separately. These results indicate that cross-talk between AM and TII cells is complex. It appears to vary with the distance and/or contact between the two cell types, and with exposure to different stimulants.

218 CYTOTOXICITY OF SIZE-SELECTED MANVILLE CODE 100 (JM-100) GLASS FIBERS ON HUMAN ALVEOLAR MACROPHAGES.

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A previous study using rat alveolar macrophages (AMs) demonstrated that glass fibers > 17μm long were larger than these pneumocytes and resulted in frustrated phagocytosis, while fibers < 7μm long were completely engulfed. Frustrated phagocytosis was associated with a substantially greater cytotoxicity of long versus short fibers (Blake et al. J Toxicol Environ Health. Part A, 54:243, 1998). Human AMs are larger than rat AMs, approximately 18 and 13μm in diameter, respectively. Therefore, the objective of this study was to evaluate the cytotoxicity of fibers of different lengths on human AMs. JM-100 glass fiber samples of 8, 10, 16, and 20μm lengths were obtained by classification of airborne fibers by dielectrophoresis. Human AMs were obtained by segmental bronchoalveolar lavage of healthy, non-smoking volunteers. AMs were treated with three different doses (determined by fiber numbers) of the sized fiber samples for 18 hours *in vitro*. Cytotoxicity caused by the fiber samples was then determined by monitoring membrane damage (leak of lactate dehydrogenase [LDH]) and loss of function (decrease in zymosan-stimulated chemiluminescence [CL]). Microscopic analysis indicated that human AMs were large enough to completely engulf fibers which were 20μm long. All fiber length fractions tested exhibited equal cytotoxicity, i.e., increasing LDH and decreasing CL in the same dose-dependent fashion. The data indicate that because human AMs are larger than rat AMs they are able to phagocytize longer fibers and the absence of frustrated phagocytosis results in lower fiber toxicity in human AMs. These differences in the AM response to long fibers between human and rat phagocytes should be considered when designing *in vivo* exposures using the rat model.

219 SILICA-INDUCED TOXICITY: *IN VITRO* AND *IN VIVO* PROTECTIVE EFFECTS OF TAURINE.

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Taurine has been suggested to have cytoprotective actions through different mechanisms including antioxidant effects. Taurine has been proposed to be a membrane stabilizer thereby preventing oxidative damage. The aim of the present studies was

to test whether taurine might act to attenuate silica-induced toxicity *in vitro* and *in vivo*. The presence of taurine resulted in a reduction of cytotoxicity with alveolar macrophages exposed to crystalline silica. To investigate further whether taurine would function effectively in the prevention of *in vivo* toxicity from exposure to silica we exposed rats by intratracheal instillation to a single dose of 5 mg silica coated with 100 mM taurine and compared the pulmonary response to rats exposed to silica or vehicle saline. Rats were sacrificed 1, 3, or 7 days postexposure, and lungs were lavaged to monitor inflammatory cells (alveolar macrophages, neutrophils, lymphocytes, eosinophils), leakage of albumin, protein and enzymes (LDH, NAG), and chemiluminescence as markers of inflammation, cytotoxicity and reactive oxygen species generated, respectively. Surface coating and coexposure of silica with taurine significantly decreased silica-induced lung injury. In conclusion, taurine showed beneficial effects in both *in vitro* and *in vivo* models of silica toxicity. This result confirms previous studies obtained in other models of lung injury.

220 ASBESTOS AND RADIATION AS COMBINED EXPOSURES IN PULMONARY FIBROSIS.

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Worker protection standards are based upon single exposures and do not account for the constellation of exposures frequently encountered in the workplace. Workers exposed to asbestos may encounter radiation as radon or from other sources. Both asbestos and radiation are etiologic agents in pulmonary fibrosis. The aim of this study is to determine whether concomitant radiation exposure in asbestos exposed workers increases the incidence of fibrosis demonstrated by International Labor Organization (ILO) opacity profusion category on chest radiographs, or spirometric evidence of restrictive disease. 1037 asbestos exposed former nuclear weapons workers from a medical surveillance program make up the study cohort. Most are male (85.5%) with an average age of 64.9 years. 663 workers had complete work histories. The demographic and fibrosis endpoints did not differ significantly between groups. Asbestos exposure based upon years in a potentially exposed job was divided into low (less than 13 years) and high (> 13 years) dose groups. 8.0% of the high dose verses 3.6% of the low dose group had ILO scores >0/1 indicating pulmonary fibrosis while 29.3% of the high dose group verses 21.8% of the low dose group have spirometry indicating restrictive disease consistent with pulmonary fibrosis. In a 2 x 4 table analysis (binary fibrosis x binary asbestos and radiation exposure) 38.6% in the high asbestos/high radiation group met the cases definition for fibrosis vs. 25.4 % in the low/low group suggesting an additive effect. This publication was prepared with the support of the US Department of Energy, under Award No. DE-FG26-00NT40938. Opinions, findings, conclusions, or recommendations expressed herein are those of the author and do not necessarily reflect the views of the DOE. Additional support: Center for Ecogenetics and Environmental Health at the University of Washington and Center for Environmental Health Sciences at the University of Montana.

221 EFFECTS OF AVASTIN™, AN ANTI-ANGIOGENIC ANTIBODY TO VASCULAR ENDOTHELIAL GROWTH FACTOR, IN A RABBIT MODEL OF VENOUS THROMBOSIS.

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Avastin™ (bevacizumab) is a recombinant humanized monoclonal antibody directed against vascular endothelial growth factor (VEGF). It blocks the interaction of VEGF with its receptors, thereby inhibiting angiogenesis. Avastin is intended as a treatment for various cancers. Several thrombotic episodes were observed in clinical trials of Avastin. This non-clinical toxicology study was performed to determine the effect of Avastin in a rabbit model of venous thrombosis. Adult male New Zealand White rabbits were given 75 mg/kg Avastin or Avastin vehicle intravenously daily for eight consecutive days. Following the final dose, a thrombus was induced in the jugular vein by application of a flow-reducing stricture proximal to the site of a clamp-induced damage. The presence or absence of occlusion was noted, as well as time to occlusion and weight of the excised clot. Cuticle bleeding time was measured. Coagulation and fibrinolysis assays were performed *ex vivo*: prothrombin time, activated partial thromboplastin time, whole blood recalcification time, activated clotting time, platelet aggregation to adenosine diphosphate, and d-dimer concentrations. Complete blood cell counts were measured. Immunohistochemical assay for von Willebrand Factor/Factor VIII complex was performed on sections of the jugular vein adjacent to the site of clot formation and on sections from the contralateral undamaged vessel. No differences were found in any of the parameters between Avastin- and vehicle-treated animals. These data suggest that rabbits subacutely treated with Avastin do not develop a prothrombotic or hyper-coaguable state in this venous thrombosis model nor is such a state demonstrable by the biomarkers measured.

222 TH9507: SAFETY STUDIES OF A GROWTH-HORMONE RELEASING FACTOR (GRF) ANALOGUE.

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TH9507 is a GRF analogue which has been stabilized by anchoring a hydrophobic moiety to the peptide, while preserving its amino acid sequence. TH9507 is in Phase II trials (doses range from 1.7 to 33.3 µg/kg) for multiple indications (muscle wasting in COPD, recovery after hip fracture surgery, sleep maintenance insomnia and immune dysfunctions). The mode of action involves induction of growth hormone (GH) release, followed by GH-induced release of Insulin-like Growth Factor–1 (IGF-1). Acute to 4-month toxicology and safety pharmacology studies have permited continuation of ongoing clinical trials, and are presented below. Acute IV studies in rats & mice at doses of 100 & 200 mg/kg produced 80% mortality at 200 mg/kg and severe but transient clinical signs at 100 mg/kg. Range-finding 2-week IV studies in rats & dogs revealed no toxicity up to the highest dose (100 µg/kg/day). IND-enabling 4-week IV studies in rats & dogs employing doses of 100 to 600 µg/kg/day revealed no adverse effects; increased body weight, adrenals weight, food intake, bilirubin, triglycerides and/or cholesterol, were attributed to the pharmacological action of TH9507. Three- and 4-month SC studies (with 1-month recoveries) in rats & dogs, respectively, employing doses of 100 to 600 µg/kg/day, revealed increased body weight, food intake, reversible hepatocellular vacuolization and/or reversible increases in phosphorus, triglycerides, cholesterol and proteins. Dose-related injection site irritancy, common to peptides, was also observed. Toxicokinetic evaluations showed a dose-related increase in TH9507 plasma levels. Bioactivity was confirmed by increased rGH and cIGF-1, in rats & dogs, respectively. In conclusion, although no dose level was free of effects, with the exception of local irritancy, all effects were judged to be due to the pharmacological activity. Furthermore, single-dose SC respiratory and CNS safety pharmacology studies in rats at doses of 0.6 to 50 mg/kg, revealed no adverse effects.

223 TWENTY-EIGHT DAY TOXICITY STUDY OF THE CANCER CHEMOPREVENTIVE AGENT 4-BROMOFLAVONE IN RATS.

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4-Bromoflavone (4BF) is being developed as a cancer chemopreventive agent. We have examined the toxicity of 4BF after 28 days of daily gavage administration to 4 groups of 20 CD® rats/sex at 0, 100, 300 and 1000mg/kg/day. No animals died. Clinical signs included dehydration and rough coat in a high dose group male (1000 mg/kg/day) and 5 males in mid dose group (300mg/kg/day) on days 1-3. Reduced total body weight gain with a corresponding reduction in food consumption was seen in all 4BF-treated groups except the low dose (100mg/kg/day) male group. Plasma levels of 4BF increased as a function of dose level, with male groups showing 2.1 & 1.5 times higher plasma drug levels than female groups at high and mid doses. Treatment-related ophthalmic lesions were absent. Non-hemolytic anemia was noted at high, mid and low dose groups due to reductions in one or more hematologic parameters (hematocrit, RBC count, MCV, hemoglobin, MCH) and increased no. of reticulocytes (high dose group only). The primary target tissues for 4BF were liver and thymus in both sexes, pituitary gland in males, and mammary gland in females. Dose-dependent hyperproteinemia, hypercholesterolemia and hypotriglceridemia were noted. These, accompanied by hepatomegaly and increased hepatocyte glycogen depletion in all 4BF-treated groups were suggestive of liver as a major target organ for the metabolic effects of 4BF rather than hepatotoxicity. Thymic lymphoid depletion in all 4BF-treated groups and hyperplasia/hypertrophy of clear cells of pituitary gland in all 4BF-treated male dose groups were also noted. Increased secretory activity of mammary glands (all doses of 4BF) and reduced weight of ovaries/fallopian tubes (at mid and high doses) were seen in female dose groups. The histopathologic changes were interpreted as related to the metabolic effects of 4BF on liver, pituitary and mammary glands (Support: NCI Contract N01-CN-85173).

224 STABILIZATION AND ANALYSIS OF PYROGALLOL (PG) IN RAT BLOOD AND IN RECEPTOR FLUID MEDIA.

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Pyrogallol, (1, 2, 3-trihydroxybenzene, PG), a metabolite of plant hydrolysable tannin gallic acid, is used in hair dyes, as a mordant in wool dying, in the manufacture of pharmaceuticals and pesticides and in topical formulations. Because of its wide