involved in the biodegradation process. Computer modeling was used to structurally characterize possible nanotube interaction sites with EPO. Studies are underway to assess oxidative biodegradation of CNT by EPO-rich activated human eosinophils. We conclude, that EPO can participate in enzymatic biodegradation of CNT after respiratory exposures during their production and handling. Supported by NIOSH OH008282; NIH NIAID U19 AI068021, HL70755, HL094488, EC-FP7-NANOMMUNE-214281

PL 55 LONG, FIBROUS CARBON NANOTUBES ACTIVATE THE NLRP3 INFLAMMASOME IN HUMAN MACROPHAGES AND INDUCE NEUTROPHILIA IN MICE LUNGS AFTER INTRATRACHEAL ADMINISTRATION.

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Carbon nanotubes (CNT) are of great interest because of their multiple applications in industry but also because of their unknown health effects. Recent studies suggest that the high aspect ratio, a feature common with asbestosis, is a key factor for reported toxicity of certain CNT. The mechanism behind this phenomenon is, however, not known. In the present study, we studied whether different carbon nanomaterials are able to induce differences in pro-inflammatory reactions in human macrophages *in vitro*. Carbon black (Evonik Industries AG); short CNT (Baytubes C150HP); long, tangled CNT (CheapTubes Inc©); long, fibrous CNT (Mitsui&Co, Ltd) and crocidolite asbestos (PRC, South-Africa) were used for *in vitro* studies. We also exposed C57BL6 mice intratracheally to fibrous and tangled CNT to investigate their effects *in vivo*. Our results showed that only long, fibrous CNT and asbestos were able to induce robust IL-1β secretion from LPS-primed macrophages. The western blot (WB) analysis confirmed that the secreted IL-1β was biologically active. Ribonucleic acid interference-mediated gene knockdown experiments demonstrated cytoplasmic NLRP3 inflammasome is essential for fibrous CNT- and asbestos-induced IL-1β secretion. Moreover, we showed that CNT-induced NLRP3 inflammasome activation is dependent on P2X7 receptor and cathepsin B activity. *in vivo* experiments demonstrated that in contrast to tangled CNT, fibrous CNT exposure elicited prominent neutrophilia accompanied by the expression of neutrophil attracting chemokines confirming our *in vitro* findings. Taken together, our results demostrate that long, fibrous CNT have asbestos-like effects being clearly more hazardous than other CNT. Fibrous CNT activated NLRP3 inflammasome causing high production of pro-inflammatory cytokine IL-1β in human macrophages. In addition, fibrous CNT exposure induced significant neutrophilia in the mouse lungs *in vivo*. Further studies are needed to make reliable risk assessment of carbon nanotubes.

PL 56 PULMONARY FIBROTIC RESPONSE TO SUB-CHRONIC MULTI-WALLED CARBON NANOTUBE EXPOSURE.

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Multi-walled carbon nanotubes (MWCNTs) are manufactured carbon compounds with many commercial applications. To address the hypothesis that MWCNTs cause persistent pulmonary pathology, C57BL/6J mice were exposed by pharyngeal aspiration to 10, 20, 40 or 80 μg MWCNTs (mean dimensions of 3.9 μm x 49 nm) or vehicle. Lungs were preserved at 1, 7, 28 and 56 days post exposure to analyze the distribution of lung burden. Morphometric measurement of Sirius Red staining was used to assess the connective tissue response. At day 1 post-exposure 62.0±2.5 and 9.9±2 percent of the lung burden (mean±SE, N=7) were in alveolar macrophages and alveolar tissue, respectively. The remainder of the lung burden (18.0±3.2) was in the airways. By 56 days post-exposure, 68.7±3.9, 7.5±1.9 and 22.0±5.1 percent of MWCNT were in alveolar macrophages, alveolar tissue and granulomatous lesions, respectively. No MWCNTs were found in the airways at 56 days. At 56 days post-exposure the average thickness of connective tissue in alveolar regions was 0.11±0.01, 0.12±.01, 0.12 ±0.01, 0.16±0.01 and 0.19±0.01 μm (mean±SE, N=6) for vehicle, 10, 20, 40 and 80 μg dose groups, respectively. The connective tissue in the alveolar region demonstrated a progressive increase in thickness over time in the 80 μg exposure group (0.11±0.01, 0.14 ±0.01, 0.16±0.01 and 0.19±0.01 μm for 1, 7, 28 and 56 day). The distribution of lung burden was predominately within alveolar macrophages with approximately 8% delivery to the alveolar tissue. Despite the relatively low fraction of the lung burden being delivered to the alveolar tissue (7.5% at day 56), the average thickness of connective tissue in the alveolar region was increased over vehicle control by 45% in the 40 μg and 72% in 80 μg exposure groups. These results demonstrate that MWCNT have the potential to produce a progressive, fibrotic response in the alveolar tissues of the lungs.

PL 57 THE ROLE OF IL-1β SIGNALING IN NICKEL ASSOCIATED MULTI-WALLED CARBON NANOTUBE-INDUCED PULMONARY INFLAMMATION.

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Exposure to certain engineered nanomaterials (ENM) has been associated with pathological changes in animal models raising concern that human health effects will emerge with increasing use. Some, but not all, ENM have been shown to activate the NLRP3 inflammasome. We have shown that nickel containing multi-walled carbon nanotubes MWCNT (Ni-MWCNT) can activate the NLRP3 inflammasome (NLRP3) in vitro using primary alveolar macrophages (AM) or THP-1 cells. Furthermore, we have also demonstrated NLRP3 activation in vitro correlates strongly with lung inflammation and pathology. Activation of caspase-1 via assembly of the NLRP3 inflammasome results in the conversion of pro-IL-1β to the active form of this proinflammatory cytokine (mature IL-1β), which is released by AM and is an important mediator of inflammation during infection. In this study, we investigated the role of IL-1β signaling to induce a pulmonary neutrophilic response using C57Bl/6 wild type or IL-1 receptor null mice (IL-1R-/-) after exposure to Ni-MWCNT. We found that Ni-MWCNT was effective in inducing pulmonary inflammation as indicated by neutrophilic influx and IL-1β secretion into the airways of wild type mice. The inflammatory response however, was abolished in mice deficient in the type I IL-1R, as indicated by significantly lower neutrophils in the inflammatory infiltrate. These data suggest an important role for IL-1β signaling in Ni-MWCNT-induced pulmonary inflammatory responses. This work was supported by NIH grants RC2-ES018742 and P20-RR017670.

PL 58 PULMONARY INFLAMMATION, EPITHELIAL HYPERPLASIA, AND LYMPH NODE TRANSLOCATION AFTER MULTI-WALLED CARBON NANOTUBE INHALATION.

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Multi-walled carbon nanotubes (MWCNTs) are engineered nanotubes with multiple fullerene carbon walls, a high aspect ratio, and rapidly increasing industrial uses. To investigate the toxicity of inhaled MWCNTs, mice were exposed 5 hours/day to 10 mg/m^3 MWCNTs (Mitsui, MWNT-7, count mode aerodynamic diameter 420 nm) for 4, 8 or 12 days and sacrificed 24 h post-exposures. Histopathologic sections of lung and tracheobronchial lymph nodes were examined at all time points and sections of nose (4 levels) were examined after the 12 day exposure. In lung, the principal changes were 1) inflammation centered around the bronchioloalveolar junction, 2) vasculitis, and 3) bronchiolar epithelial hypertrophy and hyperplasia. These were seen in all exposed mice (n=8, 6 and 6 at 4, 8 and 12 days, respectively). Peribronchiolar inflammation was principally histiocytic and neutrophilic with occasional giant cells. In many macrophages, cytopathologic changes included 1) MWCNT penetration of the cytoplasmic membrane, 2) MWCNT penetration of nuclei, and 3) karyolysis. Vascular changes were present in all exposed mice but manifestations varied and included medial hypertrophy and contraction, mural neutrophil infiltrates, and rare mural MWCNTs. Bronchiolar hypertrophy and hyperplasia were present after 4 days and persisted. After 12 days of exposure, all mice had foci of peribronchiolar fibrosis and bronchiolar epithelial mucous metaplasia. Pleural MWCNTs were seen in two mice. In lungs of air exposed controls (n=8, 6 and 6 at 4, 8 and 12 days, respectively), vasculitis and bronchiolar changes were absent; a single focus of inflammation was seen in one mouse. MWCNT translocation to the tracheobronchial lymph node progressed with time and localized to the deep paracortex, the normal location of T lymphocytes and dendritic cells. In the nose, neutrophilic rhinitis and hyaline droplet formation were consistent changes. These findings suggest that chronic inhalation toxicity studies are needed.

PL 59 UNDERSTANDING CARBON NANOTUBE GENOTOXICITY.

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Carbon nanotubes have many applications in medicine, electronics, aerospace and computer circuits. However, in order to use nanotubes for such applications, their potential genotoxic and cytotoxic effects need to be understood. We are studying nanotube interaction with cells and isolated cellular components, to determine

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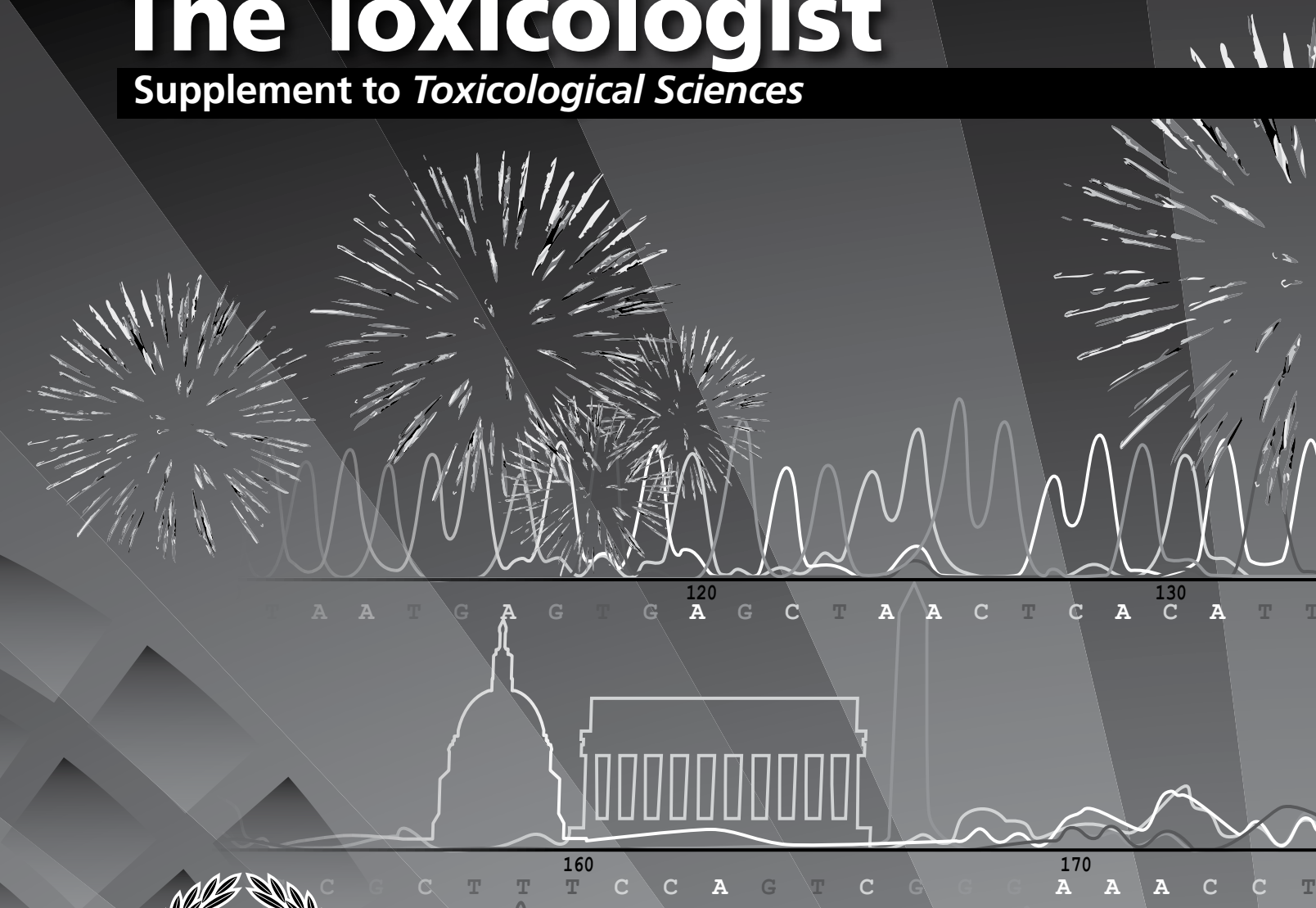

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

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