ing CF (with similar median length but different width/aspect ratio) using *in vitro* models. Sub-chronic exposures of human epithelial (BEAS-2B) and mesothelial (MET-5A) cells - both target cells of the respective lung cancer and malignant mesothelioma - to TF and CF were employed in the current study. Cells were evaluated for the presence of several cancer hallmarks indicating the neoplastic transformation following continuous exposure to the sub-toxic concentration of TF and CF for 5 weeks. TF-exposed cells, both BEAS-2B and MET-5A, revealed a neoplastic-like transformation phenotype characterized by significant increase in proliferation, morphological transformation, invasion/migration and anchorage-independent growth compared to controls. In contrast, no anchorage-independent growth was observed in CF-exposed cells although an increase in proliferation, morphological transformation and migration/invasion was detected albeit at lower intensity compared to TF. Additionally, CF had no impact on apoptosis susceptibility while TF caused increased apoptosis in MET-5A cells and its inhibition in BEAS-2B cells. Both TF and CF induced oxidative DNA damage albeit with a stronger effect in TF-exposed cells. Analysis of inflammatory responses using a cytokines/chemokines clustering approach suggested cell-type specific effects to TF and CF exposures as well as treatment related differences. Overall, our data are compatible with the interpretation that tremolite asbestos fibers demonstrated higher neoplastic transformation potential compared to CF (at the same mass dose) in both epithelial and mesothelial cells. *Disclaimer: The findings and conclusions of this report are those of the authors and do not necessarily reflect those of National Institute for Occupational Safety and Health.*

2204 Effects of Multiwalled Carbon Nanotube Accumulation on Macrophage Cell Viability and Proliferation *In Vitro*

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The range of applications for multi-walled carbon nanotubes (MWNTs), from electronics to medicine, is increasing their global production despite an incomplete understanding of their toxicological potential. A recent study reported that more than 99% of all nanoparticles administered *in vivo* are sequestered by macrophages, but the types and severity of effects induced by MWNT accumulation in macrophage cells is not well understood. Our previous work showed that macrophages preferentially accumulate ~100X more Pluronic® F108-coated carboxylated MWNTs (cMWNTs) than nonfunctionalized pristine MWNTs (pMWNTs) (Wang et. al., Nanotoxicology, 2018, DOI: 10.1080/17435390.2018.1472309). Furthermore, cMWNT accumulation in RAW 264.7 cells after a 20h exposure impaired subsequent phagocytic function. Our recent work focuses on the effects of cMWNTs on other macrophage cell functions, specifically cell proliferation and viability. To assess cell proliferation, RAW 264.7 cells were treated with varying concentrations of cMWNTs or pMWNTs and incubated at 37°C for 24, 48, or 72 hours. The cells were then washed to remove MWNTs in the media and proliferation was measured with a crystal violet assay. The results showed that neither cMWNT nor pMWNT accumulation significantly affected cell proliferation after a 24h exposure, but cell proliferation was reduced by as much as 88% and 95% after exposure to cMWNTs at 200 µg/mL for 48h and 72h, respectively. The severity by which cell proliferation decreased between 24h and 72h of exposure raised the question of whether 24h exposure to cMWNTs affected cell viability in ways undetected by the cell proliferation assay. Consequently, colony formation assays were conducted in which RAW 264.7 cells were treated either with pMWNTs at 100 or 200µg/mL, or with cMWNTs at concentrations ranging from 25 to 200µg/mL and incubated at 37°C for 24h. The cells were then washed, harvested, seeded at a density of 1000 cells per plate, and incubated at 37°C in media free of MWNTs for 10 days, after which the colonies were stained and counted. There were 371 ± 22, 248 ± 27, and 187 ± 13 colonies per plate for the control, pMWNT-treated, and cMWNT-treated cells, respectively, which suggested that cMWNT accumulation over 24h impaired cell viability by 49.6%. Additional experiments will explore the effects of cMWNTs on reactive oxygen species and apoptosis.

2205 Evaluation of Cytotoxicity Potential of 6-Thioguanine Loaded Chitosan Nanoparticles with or without Curcumin

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CCancer is the second leading cause of mortality in the world. Cancer nanotherapeutics are rapidly progressing and being implemented to overcome several limitations of conventional drug delivery systems. The objective of the study was to synthesize 6-thioguanine (6-TG) loaded chitosan nanoparticles (CNPs) and to evaluate the cytotoxicity with or without curcumin (CUR) on two cancer cell lines *viz.* Breast adenocarcinoma (MCF-7) and Ovarian teratocarcinoma (PA-1). 6-TG loaded CNPs were formulated by ionic-gelation method. Morphologically the 6-TG loaded CNPs were spherical in shape and showed mean size, PDI, zeta potential and Entrapment efficiency of 261.63 ± 6.01 nm, 0.35 ± 0.10, 15.97 ± 0.46 mV and 44.27% respectively. MTT [3-(4, 5- dimethylthiazolyl- 2)-2, 5- diphenyltetrazolium bromide] assay was conducted on MCF-7 and PA-1 cell lines at 48 h incubation for cytotoxic evaluation. IC_{50} values of 6-TG, 6-TG loaded CNPs and CUR for MCF-7 were 23.09 µM, 17.82 µM and 15.73 µM respectively. Likewise, IC_{50} values of 6-TG, 6-TG loaded CNPs and CUR for PA-1 were 5.81 µM, 3.92 µM and 12.89 µM respectively. Combination of 6-TG loaded CNPs (IC_{25}) with CUR (IC_{25}) on PA-1 and MCF-7 showed percent cell viability of 43.67 ± 0.02 and 49.77 ± 0.05 respectively. This study suggests that the cytotoxicity of 6-TG and 6-TG loaded CNPs is dose-dependent and 6-TG loaded CNPs proved to be more effective (~1.5-fold high anticancer efficacy) than that of 6-TG against PA-1 cells. Further, combination of 6-TG loaded CNPs with CUR showed synergistic cytotoxicity *i.e.* enhanced anticancer efficacy on PA-1 cancer cells.

2206 Bioactivity of Multiwalled Carbon Nanotube Mixtures with Multiple Aspect Ratios

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Multi-walled carbon nanotube (MWCNT) composites have vastly superior mechanical and structural properties compared to conventional materials. In order to be cost effective and to improve the distribution in the composite matrix, MWCNTs produced in large volume with multi-aspect ratios are utilized. Past research identified pulmonary health effects and the molecular mechanisms associated with exposure to mostly uniformly dimensional tubes; however, much is unknown concerning exposure associated with mixtures containing multi-aspect ratio tubes. In order to investigate this concern, we evaluated the toxicity profile of two multi-aspect-ratio MWCNT mixtures (MWCNT-1 and MWCNT-2) and compared them with the toxicity profile of more uniform and well-characterized MWCNT (Mitsui-7) and carbon black (CB, Printex-90) samples. Automated field emission scanning electron microscopic analysis showed that the MWCNT mixtures had a wide distribution of lengths from a few nanometers up to 20 µm and diameters that change according to length. The Mitsui-7 were more uniform with a diameter ~50 nm and the CB had a diameter of ~20 nm. Cytotoxicity and cell proliferation was assessed in human monocytic cells (THP-1) at 0 - 120 µg/ml and in primary human fibroblast cells (PHF) at 0 - 16 µg/ml. NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells)-induced inflammatory potential was screened using THP-1 reporter cells. THP-1 WT and NLRP3-deficient cells were used to screen for inflammasome activation. Acellular oxidative stress potential of the material correlated with the fold increase in *in vitro* NFκB activation and oxidative stress induction, measured using a dichlorofluorescin-diacetate assay. The extracellular remodeling and fibroblast transformation potential was evaluated by measuring collagen 1, α-smooth muscle actin, and TGF-B in PHF cells. Given the toxicity and the metrics for molecular initiating events, the MWCNT-2 mixture was the most bioactive material followed by Mitsui-7 > CB > MWCNT-1. We conclude that it was not possible to fit all multi-aspect-ratio MWCNTs into a universal toxicity profile. Ongoing extensive physiochemical characterization could elucidate the key confounders influencing the toxicity profiles of such a multi-aspect-ratio MWCNT mixture.

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