study, we observed genotoxic effects in A549 human lung cells exposed to three commercially available black toner powders. The aim of this study was to unravel the underlying mechanisms of toxicity by comparing the cytotoxic and pro-inflammatory potential of these three toner powders and the influence of ROS and NFkB induction. Toner powders A, B and C consist mainly of SAC (40–60 wt%) and magnetite (30–50 wt%). Additionally, toner powders A and B contain titanium dioxide (1–5 wt%), silica (1–5 wt%) and CB (<1 wt%). The three black toner powders and their suspensions were examined by SEM. The toner powders consist of C-bearing, rounded to slightly elongated particles with typical diameters of 2 to 8 μm. The particle surface is somewhat rough and is covered by rounded nanoparticles (30-200 nm). In all the experiments, A549 cells were exposed for 24 h to various concentrations of the three toner powder suspensions. Cytotoxicity was assessed by the WST-1 and NR assay and ROS induction by the DCFH-DA assay. Cytokine release was quantified using FlowCytomixTM Technology. Nuclear NF-kB was detected by the electrophoretic mobility shift assay (EMSA). Slight cytotoxic effects were found for the three toner powders. A concentration-dependent induction of ROS was observed upon treatment with the toner powder suspensions. Exposure to toner powder enhanced IL-6 and IL-8 production. Moreover, EMSA results exhibit release of the pro-inflammatory transcription factor NF-kB in A549 cells. These results suggest that exposure of lung cells to the three selected toner powders analyzed causes oxidative stress by induction of ROS, which may be responsible for the genotoxic effects and even trigger the pro-inflammatory processes observed.

PS 650 THE INDUCTION OF METALLOTHIONEINS BY SILVER NANOPARTICLES AS A NOVEL PUTATIVE IMMUNOMODULATORY MECHANISM IN T CELLS.

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Introduction: Silver nanoparticles (AgNPs) are used in numerous consumer and health care products, mainly for their antimicrobial properties. Recent in vitro studies suggest that AgNPs can affect the immune system. For instance, AgNPs can induce (i) DNA damage, cell cycle arrest and apoptosis in T cells (PMID 20932003), and (ii) cytotoxicity, ROS production, and IL8 secretion in macrophages (PMID 21390403). In this study we assessed whether different sizes of AgNPs have specific effects on the function of T cells in vitro. Genome wide expression patterns were used as functional readout. Methods and Results: Human Jurkat T cells (clone E6.1, ATCC) were exposed for 6 hours to three sizes of AgNPs (10, 30, 110 nm), or to a supernatant of 30nm AgNPs, that were subjected to ultracentrifugation, without or with addition of AgNO3 (0.5uM, 1.4uM), respectively. mRNA molecules were extracted and transcriptomes were generated (Affymetrix U133A, plus 2.0) from N=4 independent experiments. We found, by unsupervised hierarchical clustering of the transcriptome profiles, that metallothioneins were selectively induced by 10nm and 30 nm AgNPs, whereas 110 nm AgNPs and ionic Ag had no effects. This finding was verified by Q-RT-PCR (+2.2-26.5 fold, $p < 0.05$). Conclusions: Small sized AgNPs (10-30nm) selectively induce MTs at the mRNA level in human T cells in vitro, whereas larger AgNPs (110nm) and ionic silver do not have this property. Currently experiments are on-going to verify whether this induction of MTs (i) is also observed at the protein level, (ii) is mediated by the transcription factor MTF-1, and (iii) has specific functional consequences for the immune system.

PS 651 PULMONARY TOXICITY FOLLOWING REPEATED INTRATRACHEAL INSTILLATION OF DISPERSED SILVER NANOPARTICLES IN RATS.

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Silver nanoparticles (Ag NPs) are one of the fastest growing categories of manufactured materials in the nanotechnology industry. Our previous studies showed a dose-dependent increase in lung injury and inflammation in rats after a single bolus intratracheal instillation (IT) of 20 nm Ag NPs, persisting for 1 month post-IT at the highest dose (449 μg/rat) with resolution by 3 months post-IT. The goal of this study was to characterize pulmonary toxicity of Ag NPs after repeated lower dose IT of Ag NPs. Primary Ag NPs were 20 nm in diameter with a 0.3% wt polyvinylpovidone coating (NanoAmor, Inc.). Specific surface area was measured to be 7.54 ± 0.10 m^2/g. Ag NPs were suspended in a dispersion medium (DM, phosphate-buffered saline + 0.6 mg/ml rat serum albumin + 0.01 mg/ml dipalmitoyl phosphocholine) and sonicated. Aggregate size in DM ranged from ~ 20 to 1000 nm with an average size of 180 nm. On day 0, Sprague-Dawley rats were exposed 1x/wk for 8 wk via IT with 9.35 μg Ag NP, 112 μg Ag NP, or DM alone. Rats were humanely sacrificed 7, 28, and 84 days following the last IT, the right lung was lavaged, and the left lung was preserved for pathology analysis. Indices of lung injury (albumin and lactate dehydrogenase) were increased up to 28 days post exposure, and proteins indicative of inflammation and immune response were altered in the lavage fluid from rats exposed to the high dose. Neutrophils and lymphocytes recovered by lavage were increased in rats exposed to the high dose at all time points after treatment, with resolution of the response evident over time. Although some proteins indicative of inflammation in the lavage fluid of rats exposed to the low dose were elevated at the early time points post-exposure, there were no significant increases in lung injury parameters or cellular influx into the lungs of rats in this group. These data indicate that Ag NPs induce acute pulmonary injury and inflammation that resolves over time, unlike other toxic nanomaterials.

PS 652 PULMONARY TOXICITY ASSOCIATED WITH DIFFERENT ASPECT RATIO SILVER NANOWIRES AFTER INTRATRACHEAL INSTILLATION IN RATS.

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Respiratory exposure to nanomaterials with high aspect ratios may potentially induce greater toxicity than a low aspect ratio nanoparticle of similar composition. Production and use of silver nanomaterials is one of the fastest growing sectors in nanotechnology. The goal of this study was to characterize lung toxicity of silver nanowires (AgNWs) of varying lengths in vivo. Two AgNWs samples (nanoComposix, Inc.) ~ 50 nm in diameter, 4 μm and 20 μm long, were diluted in dispersion medium (DM; phosphate-buffered saline+0.6 mg/ml rat serum albumin+0.01 mg/ml dipalmitoyl phosphocholine). Male Sprague-Dawley rats were intratracheally-instilled with 10, 50, 125, or 500 μg of AgNWs, 500 μg α-quartz (positive control), or DM (vehicle control) on day 0. Rats were sacrificed 1, 3, and 10 days post-exposure, the right lungs were lavaged, and the left lungs were preserved for analysis of oxidative stress. Both wire samples caused a dose-dependent increase in lung injury (lactate dehydrogenase activity and albumin content in lavage fluid) and inflammation (increased lung neutrophils and phagocyte oxidant production). Both short and long wires at the 10 μg dose had no effect on lung toxicity. At the 50 and 125 μg doses, the long wires were slightly more potent than the short wires when comparing increases in lung injury. There was no difference in injury parameters comparing wires at the highest dose. However, at day 10 there was greater toxicity and less resolution of injury and inflammation in the rats exposed to 125 or 500 μg of short AgNWs compared with the equivalent doses of long AgNWs. In this study, although the longer wire caused slightly more lung injury immediately following exposure, the shorter wire induced greater toxicity over the time course; this finding may be due to greater wire number and surface area in the short wire sample when comparing samples on an equivalent mass basis.

PS 653 ADDRESSING THE TOXICOLOGICAL IMPACTS OF POLYSTYRENE AND METAL OXIDE NANOPARTICLES AS CARRIERS OF ENVIRONMENTAL POLLUTANTS.

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Nanoparticles are prevalent in the environment as engine emissions, man-made nanomaterials, etc., and can be readily found in soil and airborne. With the growing use of nanoparticles in industrial and environmental applications, the toxicological effects of these materials increasingly have come into question. Nanoparticles are thought to enter cells through active transport mechanisms such as receptor-mediated endocytosis, which has led to a growing concern over their long-term health effects.

Polychlorinated biphenyls (PCBs), which are common environmental pollutants, have well-known toxicology. PCBs can adsorb onto nanoparticles, though, primarily due to hydrophobic effects, and potentially enter cells using nanoparticles as carriers.

Various environmentally prevalent and/or toxicologically relevant PCBs, including PCB-77, -118, -126, and -153, were adsorbed onto alumina and polystyrene nanoparticles, and adsorption was confirmed using GC-MS and energy-dispersive

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