dye-based assays used to determine cell viability may produce invalid results due to CNM/dye interactions or CNM adsorption of the dye or dye products. In this study, the viability of human epidermal keratinocytes (HEK) exposed in vitro to carbon black (CB), SWCNT, and C_{60} was quantitatively determined in 96-well plates with the assays 3-[4,5-Dimethyl-2-thiazol]-2,5-diphenyl-2H-tetrazolium bromide (MTT), neutral red (NR), alamar Blue (aB), Celltiter-Blue® (CTB), Cell 96® AQueous One (CAO), CytoTox One™ (lactate dehydrogenase, LDH), calcein AM (C), and Live/Dead (L/D). In addition, trypan blue (TB) staining of HEK was quantitated with the light microscope. The assay linearity (R^2 value) was determined with HEK that were plated at concentrations ranging from 0 to 25,000 cells per well in 96-well plates. The optimal cell concentration for most of the assays, the mid point within the linear portion of the curve, was 12,500 HEK per well. HEK were then treated with 0, 0.025, 0.05, 0.1, 0.2, and 0.4 mg/ml of each CNM for 24 h. TB, C, and L/D assays, which specifically depend on direct staining of living and/or dead cells, were very difficult to interpret due to the physical interference of the CNM with the stained cells. The results of the dye-based assays vary a great deal, depending on the adsorption of the dye by the CNM. The background was much higher in the absorbance-based (MTT, NR, CAO) than the fluorescent-based assays (aB, CTB, LDH). The results of the different assays with each type of CNM was highly variable, highlighting the problem of determining accurate cell viability.

1031 INDUCTION OF AP-1-MAPKS AND NF-KB SIGNAL PATHWAYS BY TUNGSTEN CARBIDE-COBALT PARTICLES.

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Hard metal consists of a powder mixture of tungsten carbide (WC,85%) and metallic cobalt (Co,5–15%). WC–Co was evaluated as a probable carcinogen in humans by IARC in 2003. It is believed that the toxicity and carcinogenesis of WC–Co is associated with particle size. Although nanomaterials are currently being widely used in modern technology, there is a serious lack of information concerning human health effects and environmental implications of manufactured nanomaterials. In this report, we investigated the effects of nano– (40–80 nm) and fine (40 μm) 85WC/15Co particles on AP–1–mitogen–activated protein kinase (MAPKs) and NF–kB signaling in mouse epidermal (JB6) cells. The results demonstrated that the induction of AP-1 activity by nano-WC-Co was 4-times higher than that stimulated by fine–WC–Co. Similar results were obtained for NF-kB induction. The induction of AP-1 and NF–kB activity in cultured cells was time and dose-dependent. The signal transduction pathways for AP–1 activation were also investigated and the results demonstrated that both nano- and fine–WC–Co stimulate MAPK family members, including extra-cellular signal-regulated protein kinase (ERKs), p38 kinase, and C–Jun N–terminal kinase (JNKs). The potency of nano–WC-Co on MAPKs stimulation was significantly higher than that for fine WC-Co. Electron spin resonance (ESR) studies indicated that nano–WC–Co generated more reactive oxygen species (ROS) than the fine particles when incubated with the cells. Study using ROS- sensitive dyes support the conclusion that ROS are generated by WC–Co–treated cells. N–Acetylcysteine, an antioxidant agent, decreased AP–1 activation and phosphorylation of ERKs, p38 kinase and JNKs, suggest the involvement of ROS in the mechanisms of WC–Co–induced AP–1 activation. These findings demonstrate that WC–Co induces NF–kB and AP–1–MAPKs activation, which may be mediated through ROS. The size of the particles plays a critical role in toxicity and carcinogenicity of the hard metal.

1032 *IN VITRO* PENETRATION OF DENDRIMER NANOPARTICLES INTO HUMAN SKIN.

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Nanoparticles are increasingly being used in cosmetic and consumer products for the purpose of enhancing the skin penetration and stability of other ingredients. Dendrimers are spherical, highly branched, stable nanoparticles with functional groups capable of binding other molecules. Dendrimers have been shown to increase the transdermal permeation and bioavailability of indomethacin through rat skin (Chauhan et al., 2003). There is concern about the potential for nanoparticles to increase skin absorption of ingredients that are currently considered safe in cosmetics. Therefore, we evaluated the skin absorption of polyamidoamine (PAMAM) Generation 4 (G4) and Generation 5 (G5) dendrimer nanoparticles synthesized with a covalently bound fluorescent dye, fluoreseinisothiocyanate (FITC). Aqueous solutions of G4 and G5 dendrimers at concentrations of 0.08, 0.2, and 0.4%, were applied for 24 hr to human cadaver skin assembled in diffusion cells. The extent of skin penetration was determined by localization of fluorescence in the skin layers using a laser scanning confocal microscope. After 24 hr, it appeared that most of the fluorescence from dendrimers was located in or on the surface layer of skin (stratum corneum), in the opening of hair follicles, or in folds of the skin surface. At the 0.2 and 0.4% dendrimer concentration levels, even though the fluorescence appeared predominately in the stratum corneum, there was some fluorescence in the upper region of the epidermis. No fluorescence appeared in the dermis. Therefore, it appears that the G4 and G5 dendrimers can penetrate the stratum corneum and diffuse into the upper viable skin layer.

1033 INTRAVENOUS ADMINISTRATION OF GADOLINIUM MODIFIED NANOPARTICLES TO SPRAGUE-DAWLEY RATS.

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Antibody targeting of paramagnetic MRI contrast agents offers the potential of increased sensitivity by localizing deposition to the tumor site. Gadolinium (Gd)-modified alumoxane (GadAl) nanoparticles are proposed precursors that will be used in targeting tumors via antibody conjugation. In this study, we tested the general safety of GadAl compositions and their precursor nanoparticles. Adult male Sprague-Dawley rats were treated with a single intravenous infusion (IV) of the base ceramic particle, the surface modified ceramic particle and the Gd(III) modified nanoparticles (final diameters 20 and 45nm) and observed for 7 days. The base particles induced mortality immediately upon dosing. Rats treated with two 20-nm organically modified particle compositions had reduced body weight gain, but surface modification clearly resulted in increased tolerability over the base particle. Four compositions of the Gd(III) infused material, GadAl, the penultimate form prior to antibody conjugation, were tested. All 0.1 mM Gd/kg GadAl formulations (20 and 45 nm) were well tolerated with rats showing some hypoactivity after dosing and ~10% body weight gain after 7 days. Treatment with any of three different 1 mM Gd/kg GadAl formulations induced mortality immediately upon dosing whereas one 20-nm formulation was well tolerated at 0.1 and 1 mM Gd dose levels. This formulation contained the highest surface concentration of gluconic acid surface modifier and has a relaxivity approaching 30 $mM^{-1}s^{-1}$. In summary, GadAl nanoparticles and their precursors were tested for general toxicity following IV administration. Nanoparticles containing a gluconic acid modifier may be suitable for use as MRI contrast agents. Work supported by NIBIB Grant 5 R21 EB0001486-02.

1034 INDUCTION OF MATRIX METALLOPROTEINASE 2 AND 9 BY HUMAN MONOCYTES IN RESPONSE TO DIFFERENT TYPES OF METAL NANOPARTICLES.

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Recently, many studies have shown that nanoparticles can translocate from the lungs to the circulatory system. As a particulate foreign body, nanoparticles could induce host response such as reactive oxygen species (ROS) generation, inflammatory cytokines and matrix metalloproteinase (MMP) release which play a major role in tissue destruction and remodeling. MMPs are important component in many biological and pathological processes of their ability to degrade extracellular matrix components. Human MMP-2 and MMP-9 have been demonstrated to be able to degrade several substrates, including type IV collagen, elstin and native type I and II collagents. Previous studies in our laboratory have shown differences in inflammation in rat lungs after intratracheal instillation of three different kind of metal nanoparticles (Nano-Ni, Nano-Co and Nano-TiO2) that have similar mass median aerodynamic diameters and surface areas. Our previous results also suggested that different ability of metal particles in free radical generation could underlie the difference in inflammation induced by these metal nanoparticles. Here, we propose that metal nanoparticles may activate monocytes/macrophages to produce ROS and MMPs. Our results show that Nano-Co has significantly more cytotoxic effects on human monocyte cell (U937) as compared to Nano-Ni and Nano-TiO2. Nano-Co and Nano-Ni resulted in increased expression of matrix metalloproteinase 2 (MMP-2), MMP-9 and their inhibitor TIMP-2 by both zymography and RT-PCR, but Nano-TiO2 does not. Nano-Co and Nano-Ni also can stimulate ROS generation in U937 cells. Finally, Nano-Co and Nano-Ni induce HIF-α accumulation in U937 cells. These findings strongly suggest that ROS induced by metal nanoparticles is influenced by their ability to activate MMP-2 and MMP-9. This abstract is funded in part by: American Lung Association (RG-972-N), HEI (4751-RFA-05-2/06-12), IRIG grant from UofL, and 1P30ES01443-01A1 from NIEHS.

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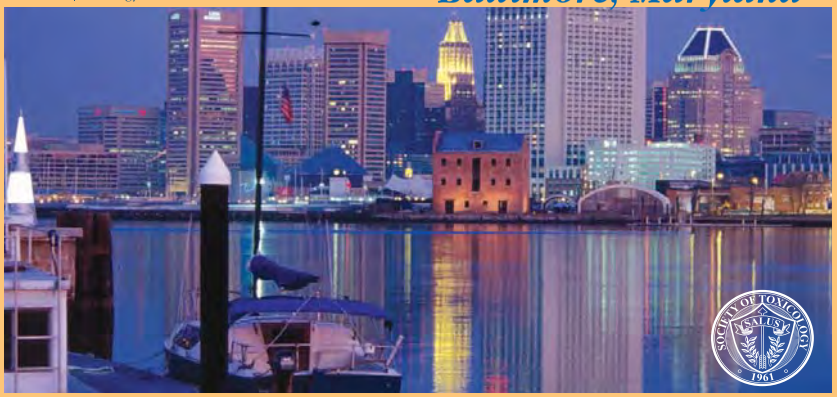

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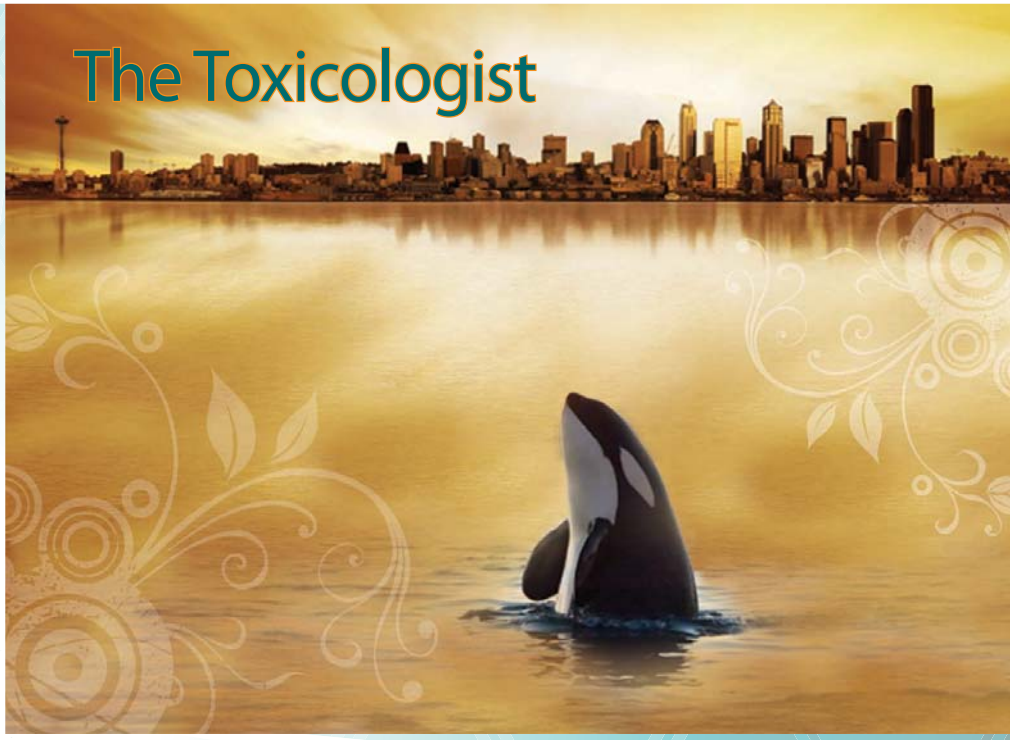

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