PS 271 SIZE-DEPENDENT EFFECTS OF TUNGSTEN CARBIDE-COBALT PARTICLES ON INDUCTION OF OXIDATIVE STRESS AND ACTIVATION OF CELL SIGNALING PATHWAYS *IN VITRO.*

A. A. Shvedova[1, 3], E. R. Kisin[1], A. R. Murray[1, 3], J. Zhao[1], L. Bowman[1], Y. Lu[1], B. Jiang[2], S. S. Leonard[1], V. Vallyathan[1], V. Castranova[1], B. Fadeel[4] and M. Ding[1]. *[1]PPRB, NIOSH, Morgantown, WV, [2]Department of Microbiology, Immunology, and Cell Biology, WVU, Morgantown, WV, [3]Department of Physiology/Pharmacology, WVU, Morgantown, WV and [4]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.*

Hard metal or cemented carbide consists of a mixture of tungsten carbide (WC) (85%) and metallic cobalt (Co) (5-15%). WC-Co is considered to be potentially carcinogenic to humans. However, no comparison of the adverse effects of nano-sized WC-Co particles is available to date. In the present study, we compared the ability of nano- and fine-sized WC-Co particles to form free radicals, and propensity to activate the transcription factors, AP-1 and NF-κB, along with stimulation of mitogen-activated protein kinase (MAPK) signaling pathways in a mouse epidermal cell line (JB6 P^+). Our results demonstrated that nano-WC-Co generated a higher level of hydroxyl radicals, induced greater oxidative stress, as evidenced by a decrease of GSH levels, and caused faster JB6 P^+ cell growth/proliferation than observed after exposure of cells to fine-WC-Co. In addition, nano-WC-Co activated AP-1 and NF-κB more efficiently in JB6 P^+ cells, as compared to fine-WC-Co. Experiments using AP-1-luciferase reporter transgenic mice confirmed the activation of AP-1 by nano-WC-Co. Nano- and fine-sized WC-Co particles also stimulated MAPKs, including ERKs, p38, and JNKs with significantly higher potency of nano-WC-Co. Overall, these findings demonstrated that both fine- and nano-sized WC-Co induce ROS generation, cell proliferation, and activation of specific cell signaling pathways. These studies also underscore that size is a critical factor in assessment of toxicological and biological responses of WC-Co materials.
Acknowledgements: supported by NIOSH OH008282, NIH HL70755, NORA 927000Y, and EC-FP-7-NANOMMUNE-214281.

PS 272 MANGANESE NANOPARTICLE CHARACTERIZATION AND POSSIBLE NEUROTOXIC MECHANISMS IN A DOPAMINERGIC NEURONAL MODEL.

H. Afeseh Ngwa[1], A. Kanthasamy[1], V. Anantharam[1], Y. Gu[2], N. Fang[2] and A. G. Kanthasamy[1]. *[1]Biomedical Sciences, Iowa Center for Advanced Neurotoxicology, Iowa State University, Ames, IA and [2]Chemistry, Iowa State University, Ames, IA.*

Chronic exposure to manganese (Mn) has been implicated in the pathogenesis of Parkinson's disease (PD). Man-made production of nanoparticles is burgeoning, increasing the probability of exposure in occupational settings. The related issue of nanoparticle toxicity has led to investigations evaluating effects of metal nanoparticles like Mn. Unfortunately, in vitro nanoparticle toxicity studies are limited by still unresolved problems relating to agglomeration and controlled dosing of nanoparticles. In this study, we systematically characterized Mn-nanoparticle size for use in N27 dopaminergic neuronal cells and then examined the metal nanoparticle-induced oxidative signaling. A combination of newly developed Differential Interference Contrast (DIC) microscopy and Transmission electron microscopy (TEM) techniques demonstrated that the Mn nanoparticles agglomerate in 10% serum RPMI media, ranging in size from single nanoparticles as small as ~ 25nM to agglomerates of up to ~ 900nM. Additional DIC studies showed that Mn nanoparticles were effectively internalized into N27 cells. Our results revealed that exposure to 25-400μg/mL Mn nanoparticles resulted in a time- and dose-dependent increase in cell death. Exposure to 50μg/mL Mn nanoparticles resulted in a significant increase in reactive oxygen species generation, accompanied by a caspase-mediated activation and proteolytic cleavage of a proapoptotic protein kinase Cδ (PKCδ). Pretreatment with caspase inhibitors blocked the induced proteolytic cleavage of PKCδ, suggesting the role of caspase-3 in kinase proteolysis. Mn nanoparticles also increased phosphorylation of PKCδ at the Threonine 505 site, suggesting the additional mode of PKCδ activation. Together, our results suggest that Mn nanoparticles of nanometer size effectively enter dopaminergic neuronal cells and can exert neurotoxic effects via activation of PKCδ, suggesting a potential to promote dopaminergic degeneration (NIH grant ES10586).

PS 273 PULMONARY TOXICITY OF INSTILLED METAL NANOPARTICLES IN THE RAT.

M. S. Horsmon[1], R. Kristovich[1], T. Taylor[2], T. Moran[1] and S. Thomson[1]. *[1]U.S. Army ECBC, Gunpowder, MD and [2]SAIC Inc., Gunpowder, MD.*

As the use of nanomaterials has become more mainstream, a fundamental understanding of the underlying toxicity of these materials is important as occupational and operational exposures become more likely. This study was designed to assess the basic pulmonary toxicity of potentially relevant brass and aluminum nanoflake particulates. Suspensions of test nanoparticles, 20-40 nm thickness and 3-5 μm in major dimension, were intratracheally instilled into the lungs of male Sprague-Dawley rats. Nanoparticles were administered in three dose groups of eight rats 0.1mg/kg, 1.0mg/kg, and 5.0mg/kg. At 24 hours and 14 days post-exposure, the rats underwent necropsy procedures involving the collection of bronchoalveolar lavage fluid (BALF) samples and fixing of the whole lung for histopathological analysis. Histopathological analysis reveals, at the 1.0mg/kg dose, brass nanoparticle exposure results in edema by 24hours post exposure and the development of fibrosis by 14 days post exposure. On the other hand, exposure to aluminum nanoparticles at 1.0mg/kg does not produce any signs of acute injury. However, macrophages are highly pigmented indicating uptake of the aluminum nanoparticles. Interleukin-1-beta (IL-1β) concentrations in the BALF of all three groups was monitored as an indicator of inflammation. At 24hours post exposure IL-1β levels in the brass 1.0 and 5.0mg/kg groups were elevated (397pg/ml and 233pg/ml respectively) with respect to the 0.1mg/kg and saline groups (25pg/ml and 2.3pg/ml respectively). By 14days post exposure IL-1β levels in all four brass groups had returned to less than 25pg/ml. At 24hours post exposure IL-1β levels in the saline, 0.1, and 1.0 mg/kg aluminum groups were all below 10pg/ml. The 5.0mg/kg aluminum group was elevated to 112pg/ml. By 14days post exposure IL-1β levels were slightly elevated in the 1.0 and 5.0mg/kg groups (48pg/ml and 83pg/ml respectively). These data support the theory that brass nanoparticles would cause acute lung toxicity whereas aluminum would produce a slower onset but longer lasting inflammatory response.

PS 274 MODULATION OF IL-8 ACTIVITY UPON CHOLESTEROL DEPLETION AND NANOPARTICLE EXPOSURE.

C. Thach and J. N. Finkelstein. *University of Rochester, Rochester, NY.*

Rationale: Cholesterol is recognized as a critical component of the plasma membrane in part as an important component of lipid microdomain. These structures are known to cluster receptors involved in chemokine production. Disruption of these membrane structures will alter cellular signaling. Nanoparticles can disrupt the lipid bilayer by creating nanoscale holes or eroding the membrane. The aim of this study is to investigate the influence of charged nanoparticles on TNF induced IL-8 expression and their interaction with lipid domains. A549 epithelial cells, containing luciferase reporter construct, were used to analyze the gene expression and production of IL-8.
Method: A549 cells were dosed with nano and micron sized cationic and anionic particles with or without methyl-β-cyclodextrin, a cholesterol chelator. An actin polymeration disruptor, cytochalasin D, was used as a control. A549 cells were treated with TNF-α for 3.5 hrs after the drug treatment. Luciferase assays and ELISAs were used to measure IL-8 activity.
Results: Cationic nanoparticles dramatically reduce IL-8 gene expression and protein level. However, the effects of cationic nanoparticles were neutralized after cholesterol depletion. Instead, there was a dramatic increase in IL-8 expression and production after methyl-β-cyclodextrin treatment. As for the micron sized cationic and anionic particles, they only reduce IL-8 protein.
Conclusion: Disruption of the membrane interferes with the effects of particles and cytokine signaling. Cationic nanoparticles require cholesterol in order to disrupt TNF induced IL-8 expression. These particles may utilize a cholesterol dependent endocytosis, possibly through lipid domains. As for the other charged particles, their mechanisms behind their impact on IL-8 activity remain unknown. Overall, it is possible that cationic and anionic nanoparticles can aggregate intracellular proteins, deterring downstream cellular signaling.

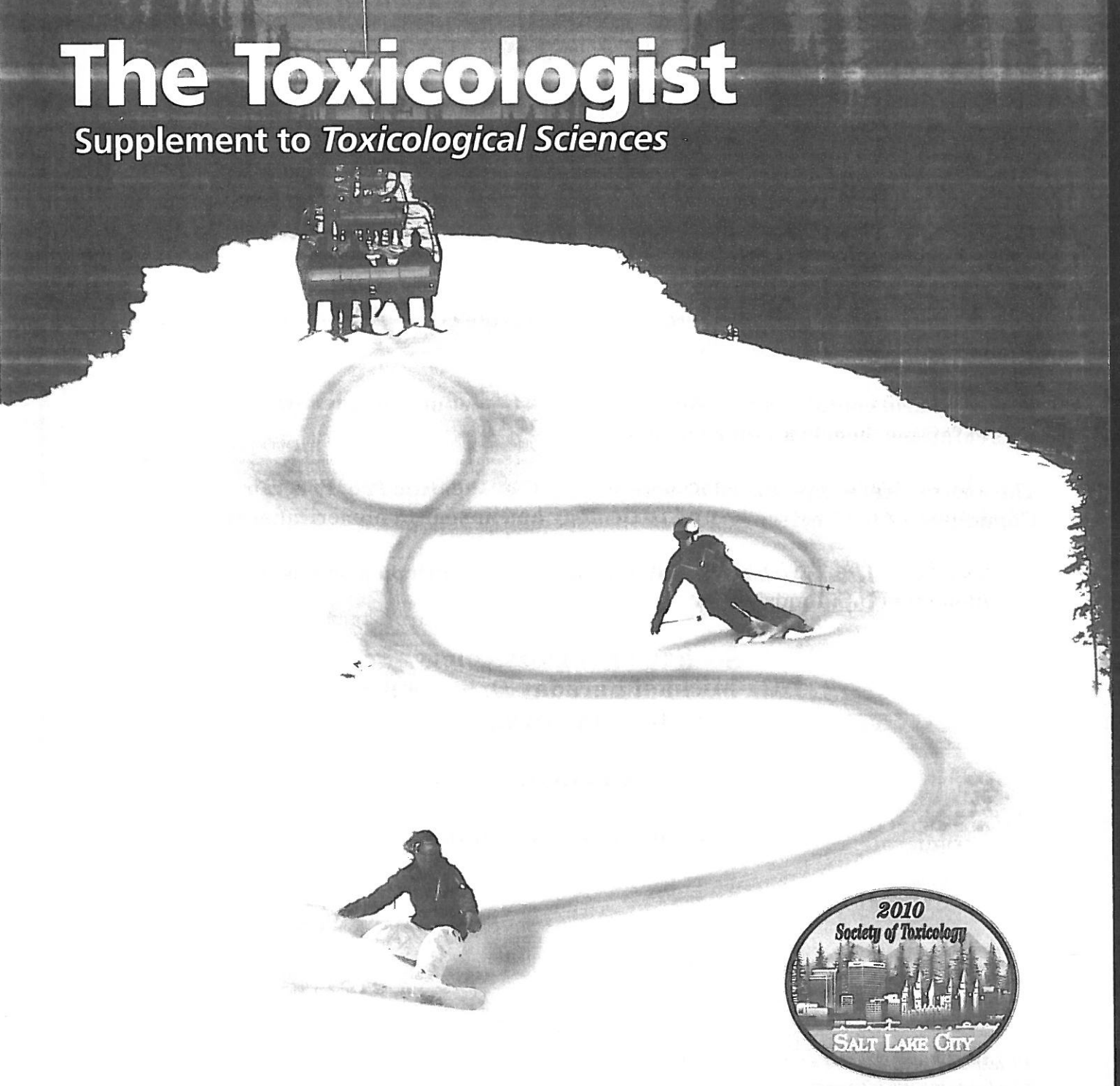
PS 275 CELLULAR RECOGNITION AND TRAFFICKING OF ANIONIC NANOPARTICLES BY MACROPHAGE SCAVENGER RECEPTOR A.

G. A. Orr[1], W. B. Chrisler[2], K. J. Cassens[1], R. Tan[2], B. J. Tarasevich[3], L. M. Markillie[2], R. C. Zangar[2] and B. Thrall[2]. *[1]Chemical Physics & Analysis, Pacific Northwest National Laboratory, Richland, WA, [2]Cell Biology & Biochemistry, Pacific Northwest National Laboratory, Richland, WA and [3]Materials Chemistry Groups, Pacific Northwest National Laboratory, Richland, WA.*

The internalization of nanoparticles (NPs) into cells is known to involve active transport mechanisms, but the precise biological molecules involved in these processes are poorly understood. We demonstrate that the level of uptake of anionic polystyrene and amorphous silica NPs (20-200 nm diameter) in a macrophage cell line is strongly inhibited by silencing expression of endogenous scavenger receptor A (SR-A), whereas NP uptake is significantly enhanced by introducing SR-A into human cells that are normally non-phagocytic. SR-A dependent NP uptake was observed both in the presence or absence of serum proteins, suggesting recognition of

The Toxicologist

Supplement to *Toxicological Sciences*



An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

49th Annual Meeting and ToxExpo™

Salt Lake City, Utah

www.toxsci.oxfordjournals.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 114, Number 1, March 2010

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 49th Annual Meeting of the Society of Toxicology, held at the Salt Palace Convention Center, March 7–11, 2010.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 473.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 496.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence.

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

www.toxicology.org