at all treatment times, except for fine-anatase after the 48-h treatment. In BEAS 2B cells, DNA damage was increased by nano-anatase after the 48-h and 72-h treatments and by nano-rutile and silica-coated nano-rutile after the 24-h and 72-h treatments. A positive result was also obtained with fine anatase at 24 h. In the CBMN assay, none of the TiO_2 types induced micronuclei. In conclusion, our results indicate that all five TiO_2 materials assayed increased DNA damage in human MET5A cells but were less effective in human BEAS 2B cells. None of the TiO_2 particle studied induced MN in BEAS 2B cells. [Funded by the European Commission (NANOSH, NMP4-CT-2006-032777)]

PS 267 DIFFERENTIAL CYTOKINE RESPONSES INDUCED BY PLAIN AND RHODAMINE-MODIFIED SILICA-NANOPARTICLES IN EPITHELIAL LUNG CELLS.

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Sponsor: M. Løvik.

Nanoparticles (NPs) of amorphous silica particles are used in a large range of products. Inhalation of such NPs may induce inflammation, and may potentially represent a health hazard as too strong and persistent inflammation is considered as a key event in development of lung disease. In this study we have investigated how modifying the particle surface may affect cellular responses. We have compared the potential of plain silica NPs (50 nm) and rhodamine-labelled silica NPs (50 nm) to induce cytokine responses in human bronchial epithelial lung cells (BEAS-2B) and in primary epithelial alveolar cells from rats. The relationship to differential activation of different signalling mechanisms (src, MAP-kinase, NFkB) and particle uptake in the cells was also examined (in BEAS-2B cells). The release of interleukin (IL)-6 and the chemokine CXCL8 (IL-8) was studied by ELISA, and the expression patterns of these cytokines were measured by real-time PCR. The importance of signalling mechanisms (p-src, p-p38, p-JNK, P-ERK, p65, and IkBα) were studied by Western analysis, and by use of different chemical inhibitors. Particle uptake was studied by confocal microscopy. The results showed that the rhodamine-labelled silica NPs induced markedly stronger IL-6 and IL-8 responses than the unmodified NPs. Similarly, the Western analysis showed most marked responses to rhodamine-labelled NPs, and in particular for p-src and p-JNK. The cytokine responses were substantially reduced by inhibition of p38 and JNK, less by inhibition of src and not by inhibition of ERK. Confocal microscopy did not show any uptake of rhodamine-labelled NPs. In conclusion, these results show that modification of the silica surface with rhodamine strongly increase the cytokine responses in epithelial lung cells, and suggest that this is related to activation of the MAP-kinases, JNK and p38, and possibly to src

PS 268 OXIDATIVE STRESS OF AMORPHOUS MONODISPERSE SILICA NANOPARTICLES IN HUMAN ENDOTHELIAL CELLS.

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The aim of this study was to investigate if oxidative stress is apparent after sub-toxic dosing of silica nanoparticles (SNP) in human endothelial cells (EAHY926 cell line). Well characterized amorphous (monodisperse) spherical SNP with a diameter of 16 and 60nm were used. Endothelial cells were incubated with nanoparticles at the concentrations from 25 to 50μg/ml and the samples were collected at different time points. Cell membrane integrity was assessed by measuring extracellular lactate dehydrogenase. The concentration of malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) was determined as markers of lipid peroxidation. HPLC–MS method was used to quantify intracellular reduced (GSH) and oxidized glutathione (GSSG). Real-Time PCR was used to assess expression of inducible genes in response to oxidative stress at 40μg/ml SNP. The significant increase of MDA and HAE concentration was observed only in cells treated with hydrogen peroxide (positive control) compared to control cells (7.7±4.3 nM/μg proteins vs 1.3 ± 0.3 nM/μg proteins, respectively). The significant increase in intracellular GSSG/GSH ratio was noticed after 1 and 4h exposure with hydrogen peroxide, and after 4h incubation with 50 μg/ml of 16nm SNP, however, already high mortality of cells was observed. Higher expression of oxidized low density lipoprotein (lectin-like) receptor 1 gene was measured in samples treated for 2, 6 and 24h with 16nm SNP whereas higher expression of heme oxygenase 1 gene was observed only after 6h treatment with 16nm SNP. The results suggest that oxidative stress is not the main mechanism contributing to cytotoxicity for the amorphous silica nanoparticles tested. Work was financed by the Belgian Ministry of Scientific Policy in the frame of S2NANO project (contract number SD/HE/02A).

PS 269 HEPATIC GRANULOMATOUS FORMATION IN NANOCERIA INFUSED RATS, IMPLICATION FOR NANOPARTICLE SAFETY.

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Objectives: We have recently reported tissue biodistribution with a relative lack of injury in male rats after acute i.v. infusion of ceria nanoparticles. In this study we examine possible long-term effects of ceria on peripheral organs and brain. Methods: Five nm ceria nanoparticles with citrate surface coating were synthesized and fully characterized for i.v. infusion at 100 mg/kg dose in male Sprague Dawley rats. After a single infusion, rats were terminated 30 days later. Multiple organs were processed for LM and TEM analysis. Other endpoints included cerium assay and oxidative stress index. Results: Overt ceria accumulation in the brain was not observed. Enlargement of spleen was apparent. The general architecture of the liver was not altered; however, granulomatous formation in hepatic parenchyma appeared in the ceria-infused rats. Histology of the nodules consisted of Kupffer cells encircled by varying amounts of mononucleated cells. Masson trichrome stain indicated no excessive collagen accumulation. The internalized ceria nanoparticles in Kupffer cells appeared as spherical to oval agglomerates of varying dimensions by TEM. Smaller, more scattered ceria aggregates were also observed in cytoplasm of the hepatocytes with many ceria clusters appearing adjacent to bile canaliculi. The latter, however, rarely contained ceria nanoparticles. Hepatocyte density showed a slight decline in comparison to the saline-infused control. Conclusion: This first report of ceria nanoparticle-induced hepatic granulomatous formation coupled with the observed splenomegaly carries considerable implication for environmental health and human safety for this type of nanoparticle; particularly in light of its extensive industrial application including the use as fuel additive and its prospective replacement for zinc oxide and titanium oxide in sunscreens. [Supported in part by U.S. EPA STAR Grant RD-833772].

PS 270 TOXIC EFFECTS OF METAL/METAL OXIDE NANOPARTICLES IN SKIN MODEL.

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Metal/metal oxide nanoparticles (Me/MeO NP), e.g. nickel (Ni), cobalt (Co), nickel oxide (NiO), and cobalt oxide (Co_3O_4), are commercially available and used by the medical/chemical industries for a number of pharmaceutical and engineering applications. The physical nature and reactive surface properties of NPs may affect their ability to induce dermal toxicity thus causing adverse skin reactions. Although the effects of Ni and Co on skin are well known (hypersensitivity, contact dermatitis and cancer), the dermal effects of Me/MeO NPs are unknown. We hypothesize that NPs may be toxic via the metal's ability to generate reactive oxygen species, initiate oxidative stress, and induce redox-sensitive transcription factors thereby affecting/leading to inflammation. Due to the skin's susceptibility to UV radiation, it is important to address the combined effect of UVB and NPs. To test the hypothesis, the effects of Me/MeO NPs (Co and Ni) were studied in vitro and in situ using murine epidermal cells (JB6 P+/+) and an engineered human skin (EpiDerm FT). Exposure of JB6 P+/+ cells to NPs resulted in the generation of hydroxyl radicals and activation of AP-1 and NF-κB. Co-exposure of JB6 P+/+ cells to UVB and NPs significantly accelerated accumulation of oxidative stress products, induced release of cytokines, cell damage and death. Co-exposure of engineered skin to NPs and UVB caused epidermal thickening, activation of dermal fibroblasts, accumulation of protein carbonyls, and pro-inflammatory cytokines. Altogether, these data indicate that co-exposure of dermal cells and engineered skin to UVB and Me/MeO NPs was associated with oxidative stress, and release of inflammatory mediators as compared to those treated with NPs alone. Therefore, it is imperative to assess the adverse effects of UVB when evaluating dermal toxicity of engineered NPs on skin.
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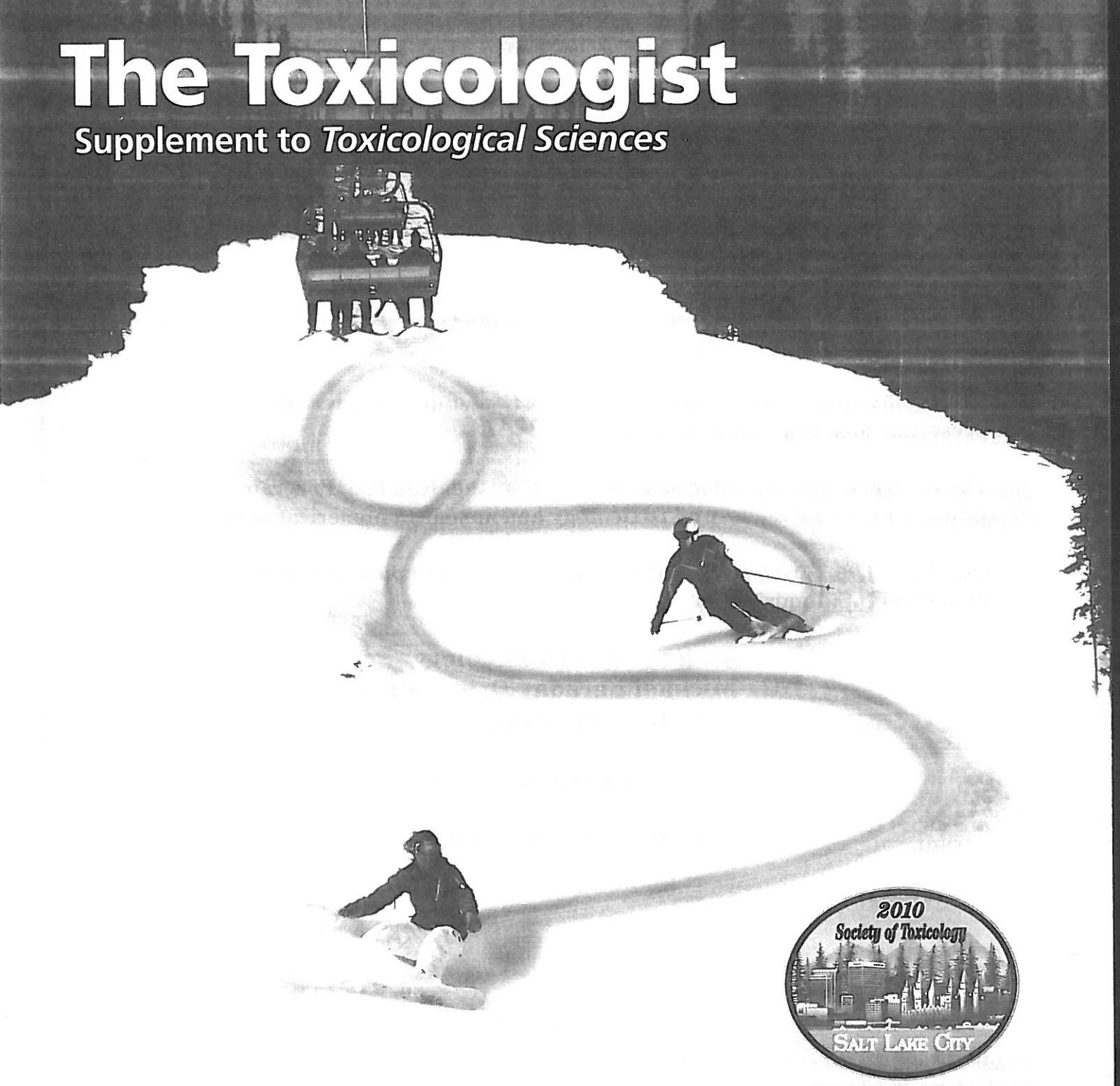

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 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.

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