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1404 SIZE- AND DOSE-DEPENDENT TOXICITY OF SIO2 NANOPARTICLES IN KERATINOCYTES.

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Although manufactured nanomaterials (NM) are becoming widely used for advancing product technology, there is a serious lack of information concerning impact on human health and the environment as large scale production, pervasive product incorporation and probable release of NM become increasingly eminent. Regarding their possible effect on cytotoxicity, and from this NM implication on human health, our main focus was to investigate eukaryotic cellular toxicity resulting from nanoparticle (NP) exposure. This study examined the cytotoxic effects of well-dispersed amorphous silica (SiO2) in mouse keratinocytes (MK). MK were exposed over a range of dose and average size distributions with homogeneous suspension of either 35, 51, 110 and 420 nm SiO2 at 0, 10, 50, 100 and 200 μg/mL for 24 h. Medium lactate dehydrogenase (LDH) leakage was dose- and size- dependent with the two smallest NP. Exposure of 35 and 51 nm at 200 μg/mL at 24 h resulted in 70% and 40% of LDH leakage, respectively. However, no changes were observed for both 110 and 420 nm NP for any concentration. Tetrazolium salt (MTT) reduction assays studying cell mitochondrial viability/function showed for 35 and 51 nm at high concentrations (200 μg/mL) produced significant toxicity compare to the larger 110 and 420 nm particles. Additional studies were carried out to investigate if redox potential of cells such (GSH/GSSG ratio) and mitochondria membrane potential as mechanism of SiO2 toxicity. GSH levels of 35 nm SiO2 at concentrations of 50, 100 and 200 ug/mL were 90, 80, and 65%, respectively. Silica nanoparticles larger than 35 nm showed no changes in GSH levels when compared with controls. Based on the results, silica NP show size- and dose-dependent toxicity.

1405 PHYSICAL CHARACTERIZATION AND DERMAL IRRITATION IN RABBITS OF TITANIUM DIOXIDE NANOPARTICLES AND NANOFIBERS.

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The field of nanotechnology has increased significantly in recent years, with a variety of applications ranging from enhanced paint pigmentation, to quantum dot semiconductors, to controlled release pharmaceuticals. With this rapid growth has also come the need to better understand the safety of nanomaterials, including nanoparticles (NP) and nanofibers (NF). The first objective of this study was to characterize submicron titanium dioxide (TiO_2) particles and fiber samples produced using a novel approach by LNK Chemsolutions. The TiO_2 NP and NF were characterized using energy-dispersive x-ray spectroscopy (EDS) and scanning/transmission electron microscopy (SEM/TEM). SEM/TEM analyses revealed that the NP were 1-2 μm diameter agglomerations of 10-100 nm spheres. SEM analysis showed the NF to be 0.5-5 μm lengths of 50-100 nm diameter cylindrical rods. The second objective of this study was to evaluate the local tolerance of TiO_2 NP and NF. New Zealand white rabbits (6/group) were administered 1 mL of TiO_2 NP and NF at 7.5 mg/mL, or 20 mg/mL for 6 hours/day for 14 days to a shaved dose site approximating 10% of the body surface area. A concurrent control group was administered the vehicle alone. Body weight, food consumption and clinical observations were performed throughout the study and an assessment of the dose site was performed daily. At the conclusion of the study the animals were euthanized, underwent a gross necropsy, and a sample of the dermal dose site was collected for histopathologic evaluation. There were no effects on body weight or food consumption and no edema or erythema was observed at any time during the study with either the NP or NF at either concentration. Additionally, there were no significant pathology findings of the dermal dose site. Based on these results, the nanomaterials were well tolerated over the 14-day study, at up to 20 mg/cm^2, the highest dose tested.

1406 SINGLE-WALLED CARBON NANOTUBES INDUCE OXIDATIVE STRESS AND INFLAMMATION IN SKIN.

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Single-walled carbon nanotubes (SWCNT) are a novel material with unique electronic and mechanical properties. A variety of different techniques are available for the production of SWCNT; however, the most common is via the disproportionation of gaseous carbon molecules supported on catalytic iron particles (HiPco). The SWCNT produced by this method usually contain significant amounts of iron that may act as a catalyst of oxidative stress. The skin is a prime target for SWCNT toxicity as topical exposure can result during technologic processing and use. We hypothesized that SWCNT are toxic to the skin and the toxicity is dependent on their content of iron. The major toxicity mechanisms include induction of an inflammatory response, and oxidative stress exacerbated by iron. To test this hypothesis, the effects of SWCNT were assessed *in vitro* and *in vivo*. Exposure of human keratinocytes (HaCaT) revealed cytotoxicity in cells exposed to SWCNT; partially-purified SWCNT (0.25 weight % iron) exerted lower toxicity than unpurified SWCNT (40 weight % iron). Murine epidermal cells (JB6 P+) revealed a significant dose-dependent activation of AP-1 following exposure to unpurified SWCNT while partially-purified SWCNT did not activate AP-1. NF-κB was dose-dependently activated by both unpurified and partially-purified SWCNT. *In vivo* experiments evaluated the skin of SKH-1 mice following 5 days of unpurified SWCNT exposure (2, 4, or 8 mg/kg). Depletion of glutathione, increased myeloperoxidase activity, and an increase in IL-6 levels were observed following exposure to unpurified SWCNT. Histological evaluation of the skin following SWCNT exposure revealed an increased number of mast cells and polymorphonuclear leukocytes around hair follicles of the mouse skin. These data indicate that dermal exposure to SWCNT, particularly unpurified SWCNT, can result in inflammation, oxidative stress and dermal toxicity during occupational exposures.

1407 ANTAGONISM OF THE ARYL HYDROCARBON RECEPTOR.

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The aryl hydrocarbon receptor (AHR) binds to a variety of ligands and subsequently controls transcription of target genes, which mediate the toxic or carcinogenic effects of these compounds. Many AHR agonists and partial agonists are known to induce transcription; however, a pure antagonist of the AHR has not been discovered. This ideal pure antagonist would competitively inhibit the effects of a potent AHR agonist, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Previous reports indicate that 3'-methoxy-4'-nitroflavone (MNF), 3,3'-diindolylmethane (DIM), 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), α-naphthoflavone (ANF), salicylamide (SAL), and resveratrol (RES) are potential antagonists. To measure the antagonistic properties of these compounds, we performed dose-response experiments with TCDD and the potential antagonists in H1G1 mouse hepatoma cells, which stably express green fluorescent protein regulated by AHR-responsive elements. We also tested these compounds' induction of endogenous CYP1A1 and CYP1B1 mRNA expression in H1G1 cells. ANF and RES induced some expression of both the reporter and endogenous genes and did not effectively antagonize TCDD's induction of the reporter or endogenous genes. MCDF and MNF acted as partial agonists by inducing some expression and inhibiting TCDD's induction of the reporter and endogenous genes. While both DIM and SAL were effective antagonists of the reporter gene, SAL did not effectively antagonize TCDD's induction of the endogenous genes. Only DIM potently antagonized TCDD's induction of the endogenous genes. Next, we measured the ligands' regulation of CYP1A1 and CYP1B1 mRNA expression in MCF-7 breast cancer cells. The efficacy of the compounds in the two cell types differed, suggesting the efficacy of AHR ligands could be species- and/or tissue- specific. Further research should investigate the mechanisms of the differences between the induction of endogenous and reporter genes in H1G1 cells and between the induction of the endogenous genes in H1G1 and MCF-7 cells.

1408 REGULATION OF GENE EXPRESSION BY THE ARYL HYDROCARBON RECEPTOR IN BREAST TUMOR CELL LINES.

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Previous reports have shown differences between estrogen receptor (ER)-positive and ER-negative breast cancer cells in regulation of gene expression by the aryl hydrocarbon receptor (AHR). We compared immortal (MCF-10F) and transformed (BP-1) ER-negative cells as well as ER-positive MCF-7 cells. While previous studies have found that AHR agonists do not induce cytochrome P450 1A1 (CYP1A1) expression in ER-negative cell lines, we found that expression of CYP1A1 and cytochrome P450 1B1 (CYP1B1) mRNAs is induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in MCF-10F cells, BP-1 cells, and MCF-7 cells. In contrast, 3'-methoxy,4'-nitroflavone (MNF), an AHR antagonist, does not increase mRNA expression of CYP1A1 or CYP1B1 in MCF-10F or MCF-7 cells, but it does increase CYP1A1 and CYP1B1 expression in BP-1 cells. Chromatin immunoprecipitation assays showed increased occupancy of CYP1A1 and CYP1B1 regulatory regions by AHR in both MCF-10F and BP-1 cells treated with TCDD. This increased promoter occupancy was not found in MCF-10F cells treated with MNF. We conclude that gene expression and promoter occupancy in response to AHR ligands are dependent upon differences among breast cell phenotypes that are not limited to ER status.

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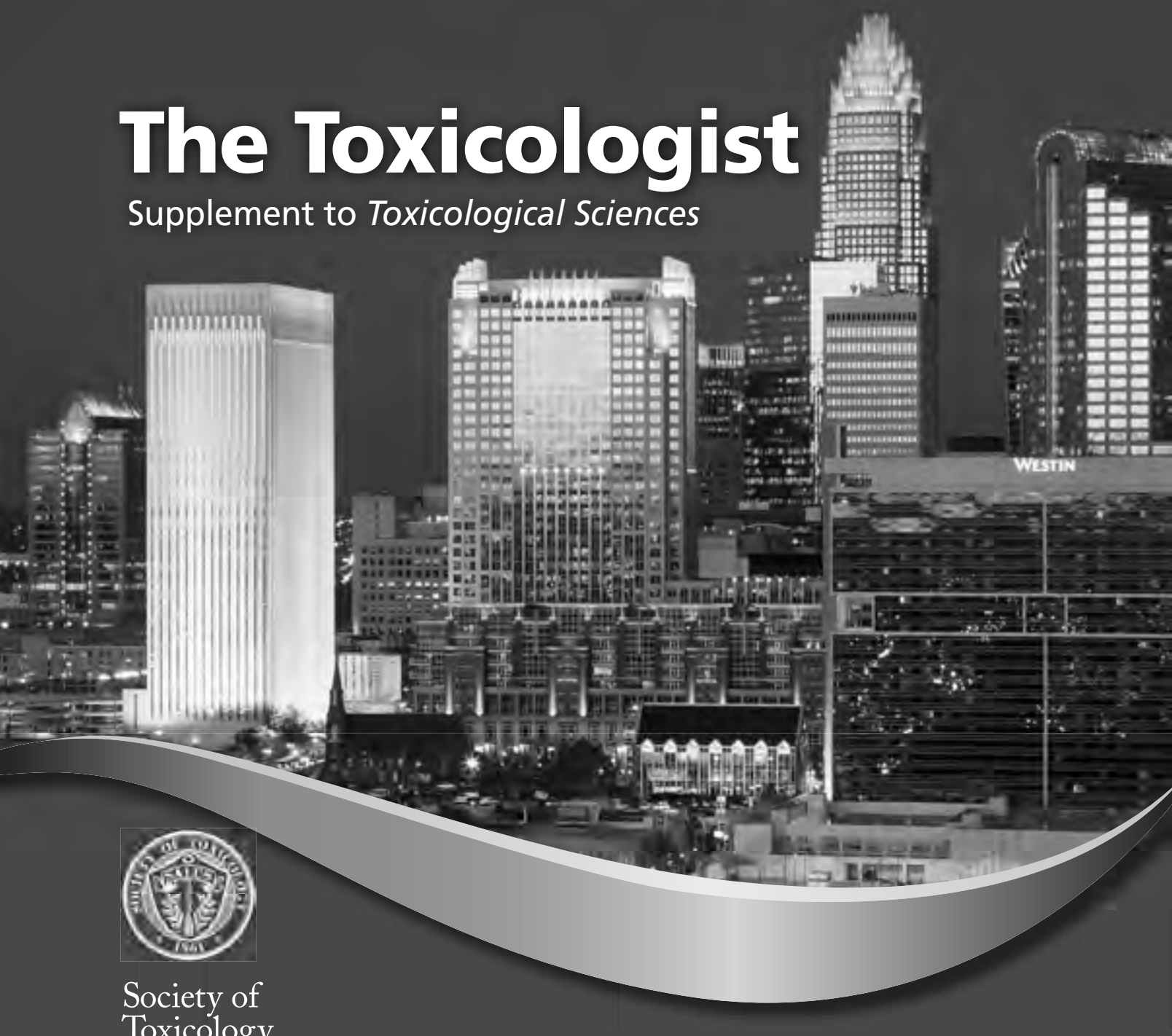

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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

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