decision making. In this project, we focus on the evaluation of the pro-fibrotic activities of NM and develop advanced *in vitro* models for the assessment of NM-induced lung fibrosis. Based on the central role of fibroblasts in lung fibrosis, we developed a strategy of fibroblast exposure mimicking the pulmonary environment of this cell type during inhalation exposure by assessing the responses to a direct contact of fibroblasts with NM, or indirect effects of NM on fibroblasts via their crosstalk with epithelial and inflammatory (mainly macrophages) cells. Pro-fibrotic responses to NM are examined in human lung fibroblast cell lines (MRC-5 and CRL1490) acutely (24 h) and chronically (8 weeks) exposed to low (realistic) doses of NM. Fibroblasts will also be treated with supernatants of epithelial and inflammatory cells (cultured alone or in co-culture) acutely or chronically exposed to NM to mimic the indirect activity of NM. Acute and chronic, as well as direct and indirect effects, will be compared to identify the modes of action of the tested NM. As we previously identified the activation of fibroblasts as a relevant key event with a high predictive value for lung fibrosis [1], we will focus on fibroblast proliferation, differentiation, collagen production, etc. The predictive value of the different models will be evaluated by comparing NM able to induce lung fibrosis *in vivo* (e.g. multi-wall carbon nanotubes, MWCNT) to non-fibrotic NM (e.g $BaSO_4$). High aspect ratio NM (MWCNT) will also be compared to low aspect ratio NM able to induce lung fibrosis (e.g. $CeO_2$). *In vitro* models and conditions are currently under development and preliminary results will be presented. 1. Vietti, G., D. Lison, and S. van den Brule, *Mechanisms of lung fibrosis induced by carbon nanotubes: towards an Adverse Outcome Pathway (AOP).* Part Fibre. Toxicol, 2016. 13(1): p. 11.

## 2127 Cytotoxicity of Engineered Nanomaterials on Primary Epithelial Cells in Air-Liquid Interface Culture

L. M. Tran, J. Bustamante, K. J. Mo, S. M. Thomasy, K. E. Pinkerton, and L. S. Van Winkle. *University of California Davis, Davis, CA.*

The unique physicochemical characteristics of engineered nanomaterials (ENMs) have led to their increased applications in pharmaceuticals, biomedical devices, and consumer products. ENMs range from 1-100 nm in at least one dimension and possess unique properties at an atomic level. They are often used to increase the quality of consumer products due to their stability, thermal conductivity, and mechanical properties and exhibit different properties depending on their size and surface area. Graphene is known for its optical transmittance and chemical inertness; graphene oxide and reduced graphene oxide nanoparticles have been incorporated in biomedicine for biosensing and drug delivery. Copper oxide nanoparticles exhibit high thermal conductivity, stability and potentially antimicrobial properties. The versatility and increasing use of ENMs raises concerns for human health since inhalation can be a major route of exposure with potential mechanisms of toxicity not well understood. Because ENMs vary in size, structure, and chemical properties, it is difficult to assess which characteristic is associated with biological toxicity. In order to shed light on the role of different physicochemical properties in respiratory toxicology *in vitro*, we exposed primary mouse tracheal epithelial cells on an air liquid interface (ALI) culture to well characterized ENMs of different chemical properties and sizes. These ENMs include: copper oxide, cadmium sulfide, molybdenum disulfide, hexagonal boron, graphene oxide in water, graphene oxide (250 nm), graphene (110 nm), and reduced graphene oxide. Cells were grown in culture until confluent and fully differentiated, then exposed to ENMs at concentrations of 125 or 250 µg/mL for 24 hours. Because assays for measuring cell number metabolically are prone to particle interference, cytotoxicity was determined by fluorescent microscopy on transwell membranes. Our results showed that copper oxide, hexagonal boron, graphene (110 nm), and reduced graphene oxide at a concentration of 250 µg/mL were cytotoxic as well as graphene oxide in water at 125 µg/mL. The remaining ENMs tested did not cause cytotoxicity. We conclude that this method can be used to study ENM toxicity and that copper oxide nanoparticles are cytotoxic to large airway epithelial cells. *Supported by U01 ES027288.*

## 2128 Exposure to Nanoparticles in a Food Matrix Alters Intestinal Function in an *In Vitro* Model

M. Malik, C. N. Marques, and G. Mahler. *Binghamton University, Binghamton, NY.*

Overall well-being is related to gut health and function. The gastrointestinal (GI) tract forms a selective barrier that protects the host from harmful luminal content and allows essential nutrients and water to pass into circulation. Food additives and microbial dysbiosis may influence intestinal health and activity of intestinal alkaline phosphatase (IAP), a gut mucosal defence factor, while playing a role in the development of GI disorders. An average of $10^{12}$-$10^{14}$ edible metal oxide nanoparticles (NP) are consumed per day. In this study, a Caco-2 and HT29-MTX E12 intestinal model of the GI tract was used to study the effects of food-grade titanium dioxide NP in a food matrix (FM) on intestinal permeability, IAP activity, and nutrient transport. The NP-microbiota-gut interaction was tested by including Gram-positive, commensal (*LactoBacillus rhamnosus*, *Bifidobacterium bifidum*) or Gram-negative, opportunistic (*Escherichia coli*) bacteria into the model. The model was subjected to $10^3$ CFU/mL of bacteria and physiological doses of digested $TiO_2$ NP in FM. The FM was a semi-synthetic meal prepared with all the essential components of a regular diet. Cell membrane permeability was assessed by a Lucifer Yellow (LY) permeability assay. Glucose, protein and fatty acid transport were assessed using colorimetric assays. Post-exposure, bacterial cell viability was assessed with a drop plate method. While FM did not affect the barrier integrity, *E. coli*+$TiO_2$ NP in FM showed a decrease in permeability ($p<0.0001$) and IAP activity ($p<0.001$) compared to no bacteria condition. $TiO_2$ in FM led to a decrease in protein and triglyceride transport across the barrier. *E. coli* remediated this effect of NP. Glucose transport was unaffected by the NP. A permeable intestinal barrier can lead to systemic inflammation and potential tissue damage. The results of this study suggest that while $TiO_2$ affects protein transport, *E. coli* can ameliorate the changes. Dietary conditions influence the attachment of bacterial cells to the cell monolayer, further affecting the intestinal function. These results highlight the complex interaction of the microbiome, diet, and gut function. With the use of multiple human intestinal cell types, human-derived bacteria and digestion, our *in vitro* model provides a physiologically relevant method to investigate these interactions.

## 2129 Mechanisms of Rare Earth Metal Oxide Nanoparticle Toxicity in Macrophages

K. L. Rogers, K. N. Nguyen, and J. M. Brown. *University of Colorado Anschutz Medical Campus, Aurora, CO.*

Rare earth metal oxide nanoparticles (REMO NPs) are synthesized from lanthanide series metals complexed with oxygen. REMO NPs are desired due to their unique chemical, catalytic, electrical, magnetic, and luminescent properties for applications in both material science and medicine. Occupational exposure to REMO NPs may occur through inhalation during their manufacturing. Additionally, biomedical use of these nanoparticles is increasing therefore it is important to understand their toxicity. Further, due to their novelty, the toxicity of these particles is not well characterized. Recently, several studies have shown adverse effects associated with exposure to REMO NPs including pulmonary inflammation *in vivo* and cytotoxicity *in vitro*. We hypothesized that toxicity of REMO NPs is due to their unique physicochemical properties and would elicit adverse immune responses in macrophages. Ten different REMO NPs were screened for cytotoxic effects by dosing the RAW 264.7 macrophage cell line with 1-50 µg/mL for 24 hours. Four nanoparticle types were selected for further testing and characterization based on differences in cytotoxicity profiles. These four were lanthanum oxide ($La_2O_3$) (high toxicity), gadolinium oxide ($Gd_2O_3$) (medium toxicity), neodymium oxide ($Nd_2O_3$) (medium toxicity), and europium oxide ($Eu_2O_3$) (low toxicity). Using these four REMO NPs, we further investigated the correlation of physicochemical properties to cytotoxicity and activation of macrophages including reactive oxygen species generation and cytokine production. It was found that hydrodynamic size and the amount of REMO NP uptake by macrophages directly correlated to cytotoxicity, mitochondrial disfunction, and inflammatory responses, thus, linking the physicochemical properties of REMO NPs to their effect on macrophages. In conclusion, the safety profile of REMO NPs should be further evaluated prior to incorporation in biomedical applications.

## 2130 Disruption of Bronchial Cell Monolayer Integrity by Organomodified Nanoclays and Their Incinerated Byproducts

T. Stueckle[1], A. White[2], A. Wagner[2], R. Gupta[2], Y. Rojanasakul[2], and C. Dinu[2]. *[1]NIOSH, Morgantown, WV; and [2]West Virginia University, Morgantown, WV.*

Organomodified nanoclays (ONCs) represent one of the most used engineered nanomaterials (ENMs) as nanofiller in emerging advanced manufacturing strategies to produce a diverse number of polymer nanocomposites. Different organic quaternary ammonium coatings on these 2-dimensional montmorillonite nanoclays allow for their incorporation in novel or replacement technologies in thin-film, aerospace, automobile, consumer, and health care polymer nanocomposite applications. Compared with other ENMs, little information exists on risks to occupational pulmonary health along the ONC life cycle that encompass synthesis, handling, manipulation, and disposal. This study hypothesized that coating type, incineration status, and time-dependent effects of ONC exposure would impact bronchial epithelial cell monolayer integrity, a key target following inhalation exposure. High-throughput *in vitro* screening strategies including high content imaging, electric cell impedance sensing, and flow cytometry were employed to evaluate a

set of pre- and post-incinerated ONCs for acute effects and fate of the monolayer post-exposure. Using each particle's $IC_{50}$ cell viability in a BEAS-2B cell model, pristine nanoclay exposure caused acute loss of monolayer integrity, decreased metabolism, and increased apoptosis. Three different ONCs, however, displayed minimal loss to monolayer integrity despite coating type-dependent differences in apoptosis induction and decreased cell metabolism. Conversely, incinerated nanoclay byproducts caused decreased monolayer integrity, increased cell necrosis, and little evidence for reestablishment of the epithelial monolayer. These results suggest the type of quaternary ammonium coating and incineration status largely impacts mechanism of cytotoxicity, cell metabolism, and the recovery ability of the exposed bronchial epithelial cell monolayer. An integrated high-throughput *in vitro* screening strategy, using high content imaging and traditional *in vitro* methods, represents a rapid pulmonary epithelial toxicity assessment approach to prioritize ENMs for further evaluation and serves to inform 'prevention-by-design' material development strategies.

## PS 2131 Selective Uptake of Carboxylated Multiwalled Carbon Nanotubes via Class A Type 1 Scavenger Receptors Impairs Viability and Phagocytic Activity in Macrophages

R. Wang, E. Chow, R. Lohray, P. Gangupantula, L. L. Smith, and R. Draper. *University of Texas at Dallas, Richardson, TX.*

Class A type 1 scavenger receptors (SR-A1) are involved in a variety of immunological and pathological responses. Our published work suggested that SR-A1 may be involved in the marked accumulation by cells of carboxylated multi-walled carbon nanotubes (C-MWNTs) compared to pristine MWNTs (P-MWNTs) (Wang et. al., *Nanotoxicology*, 2018). The studies in this poster confirm and extend the role of SR-A1 in mediating C-MWNT uptake. 1. Selective C-MWNTs accumulation correlates with surface SR-A1 receptor expression. RAW 264.7 and wild type B6 macrophages, which express high SR-A1 levels, accumulated high amounts of C-MWNTs, but not P-MWNTs or amino-functionalized MWNTs (N-MWNTs) at 37 °C. However, ZK macrophages, which are genetically deficient in SR-A1 receptors, did not accumulate significant amounts of C-MWNTs above background level. Further, stably transfected CHO cells that express mouse SR-A1 receptors had increased uptake of C-MWNTs compared to untransfected controls. 2. C-MWNT accumulation impairs phagocytic function via SR-A1 receptors. The physiological impact of C-MWNTs on subsequent phagocytosis of ligands that are known to interact with SR-A1 receptors was determined using confocal fluorescence microscopy and flow cytometry. There were significant reductions in the uptake of polystyrene beads and *E. coli* by RAW 264.7 cells pre-treated with C-MWNTs, but not with P- or N-MWNTs. The study on oxLDL uptake is underway. Further, the 24h continuous exposure of RAW 264.7 cells to C-MWNTs reduced surface SR-A1 receptors by almost 50%. 3. C-MWNT accumulation impairs macrophage viability. The impact of C-MWNTs on the proliferation of RAW 264.7 cells was assessed using a crystal violet assay and an 8d colony formation efficiency (CFE) assay. The accumulation of C-MWNTs impaired cell proliferation with IC50 values of 120 and 80 µg/mL for 48 and 72h exposure, respectively. P- and N-MWNTs have minimal effect on cell proliferation. In CFE assays, C-MWNTs reduced colony formation during an 8 day exposure with and IC50 of 29 µg/mL. These results confirmed that SR-A1 significantly contributes to the selective uptake of C-MWNTs and that SR-A1-mediated uptake of C-MWNTs impaired SR-A1-dependent phagocytic activities in macrophages and reduced cell viability.

## PS 2132 Effect of Titanium Dioxide Nanoparticles on DNA Methylation in Human Cells

M. Pogribna, N. Koonce, A. Mathew, B. Word, A. K. Patri, B. D. Lyn-Cook, and G. J. Hammons. *US FDA/NCTR, Jefferson, AR.* Sponsor: I. Pogribny

The dramatically increased use of nanomaterials, including nanoscale titanium dioxide ($TiO_2$), raised the concern of their potential risk to human health. The present study aimed to further the understanding of the underlying mechanism of $TiO_2$ nanoparticles toxicity by examining the time-dependent alterations in cytosine DNA methylation induced by $TiO_2$ nanoparticles exposure *in vitro*. Human skin (A-431), lung (NL20), liver (HepG2), and colon (Caco-2) cells were treated with $TiO_2$ nanoparticles at the sub-cytotoxic doses for 24 and 72 hours. Treatment with $TiO_2$ nanoparticles resulted in global and gene-specific cytosine DNA methylation alterations. In particular, global DNA methylation decreased in three cell lines (Caco-2, HepG2, and A-431), while across the four examined cell lines, eight genes indicative of cellular stress response and toxicity (CDKN1A, DNAJC15, GADD45A, GDF15, INSIG1, SCARA3, TP53, and BNIP3) exhibited increased DNA methylation. Additionally, treatment with $TiO_2$ nanoparticles altered the expression of genes involved in establishing and maintaining DNA methylation patterns (DNMT1, DNMT3A, DNMT3B, MBD2, and UHRF) in cell-type- and time-dependent manner, with the greatest effects being found in NL20 and A-431 cells. The results of this study demonstrate that the sub-cytotoxic concentrations of $TiO_2$ nanoparticles induced treatment-related cytosine DNA methylation changes, indicating the potential value of epigenetic evaluation in the toxicity assessment of nanoparticles.

## PS 2133 Survival Mechanisms in Keratinocytes Exposed to Subtoxic Concentrations of Metal-Derived Nanoparticles and Their Susceptibility to Xenobiotic Exposure

V. Montesinos[1], J. Rose[2], A. De Vizcaya[1], and R. Franco[2]. *[1]CINVESTAV, Mexico, Mexico; and [2]University of Nebraska, Lincoln, Nebraska, NE.*

Metal-derived nanoparticles (Mt-NPs) are increasingly used in cosmetology due to their ultraviolet shielding (titanium dioxide [$TiO_2$]), antioxidant (cerium dioxide [$CeO_2$]), and/or biocidal (silver [Ag]) properties. In the absence of overt toxicity (i.e. cell death), Mt-NPs are considered safe for their use in cosmetology. However, there is little understanding about the mechanisms involved in the survival of keratinocytes exposed to subtoxic levels of Mt-NPs, an issue that we aimed to address in this study. Human keratinocytes (HACAT) were exposed subacutely (48-72 h) to subtoxic concentrations (≤30 µg/ml), of rutile (r) $TiO_2$ (cylindrical), $CeO_2$ (cubic) and Ag (spherical) with a core / hydrodynamic size of <40 / <100 nm and >98% purity. Mt-NP uptake was quantified by changes in the light side scatter (SSC), where the kinetics (time-dose-response) suggested that they were internalized to a similarly extent by keratinocytes. r$TiO_2$ and $CeO_2$, but not Ag NPs, increased autophagy, and inhibition of autophagy flux (chloroquine) and autophagosome elongation (3-methyladenine) prompted cell death. In contrast, no increase in the steady-state levels of reactive oxygen species (ROS) was induced by exposure to any of the Mt-NPs tested. Interestingly, intracellular Ag aggregates observed a far-red autofluorescence (≥740 nm em), which has been ascribed to their binding to thiol molecules such as glutathione (GSH). Accordingly, inhibition of GSH synthesis with buthionine sulfoximine sensitized keratinocytes to Ag, but 6-aminonicotinamide, which impairs the recycling of oxidized GSH, had no effect on cell viability. r$TiO_2$ and Ag, compromised metabolic flux (glycolysis and mitochondrial respiration), but ATP levels were unaltered. Finally, we observed that Mt-NPs sensitized keratinocytes to non-UV xenobiotics (arsenite and paraquat). Our results demonstrate the differential contribution of autophagy and GSH metabolism to the survival of keratinocytes exposed to subtoxic concentrations of Mt-NPs, and highlight the increased susceptibility of keratinocytes exposed to Mt-NPs to xenobiotic exposure.

## PS 2134 The ToxTracker Reporter Assay as a Tool for Mechanism-Based (Geno)toxicity Screening of Nanoparticles: Metals, Oxides, and Quantum Dots

S. McCarrick[1], F. Cappellini[1], N. Moelijker[2], R. Derr[2], G. Hendriks[2], and H. L. Karlsson[1]. *[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; and [2]Toxys B.V., Leiden, Netherlands.*

The increased manufacturing and use of nanoparticles (NPs) require faster and more efficient testing of their potential toxic effects. Traditional *in vitro* genotoxicity assays are often time-consuming and have the limitation that they generally lack the ability to provide insight into the mechanisms. One alternative approach is the use of reporter cell lines to quickly assess the cellular stress response pathways that are activated after exposure to NPs. In this study the applicability of the ToxTracker reporter cell lines to identify the (geno)toxicity of various metallic- or metal oxide NPs (n=18) including quantum dots (QDs) of various sizes was explored. The ToxTracker results were compared with historical genotoxicity results and physico-chemical characterization of the various nanomaterials. The results show a large variation in cytotoxicity of the tested NPs. Furthermore, the primarily effect observed in ToxTracker was activation of the oxidative stress reporters although some NPs also induced the reporters for DNA damage. Some NPs were non-cytotoxic and did not cause a clear activation of any of the reporter cell lines (Au, Cr, $Cr_2O_3$, Pt, $SnO_2$ and V). Other NPs were highly toxic e.g. antimony (Sb) NPs causing cytotoxicity and activation of reporters at relatively low doses (<2µg/ml). These effects were also observed for $Sb_2O_3$ but at much higher doses. NPs of manganese (Mn and $Mn_3O_4$) induced the most remarkable response in the ToxTracker assay with induction of reporters for oxidative stress, DNA damage, protein unfolding and p53-related stress. The CdTe QDs were also highly toxic showing clearly size-dependent effects and calculations suggest that surface area is the most relevant dose metric. Of all metal- and metal oxide NPs investigated (n=33), CuO, Co, CoO, CdTe QDs, Mn, $Mn_3O_4$, $V_2O_5$, and welding NPs clearly induced the Rtkn DNA damage reporter (> 2-fold). Also, NPs of Ni, NiO and $Cr_2O_3$ induced a weak response of this reporter (>1.5

SOT 2020

59th Annual Meeting & ToxExpo

Anaheim, California • March 15–19

# The Toxicologist

## Supplement to *Toxicological Sciences*


Toxicological Sciences

ISSN 1096-6080
Volume 174, Issue 1
March 2020

The Official Journal of the Society of Toxicology

OXFORD UNIVERSITY PRESS

SOT | Society of Toxicology

www.academic.oup.com/toxsci

Publication Date: February 21, 2020

# Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and Scientific Sessions of the 59th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 15–19, 2020.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 542.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 580.

The abstracts are reproduced as accepted by the Scientific Program Committee of the Society of Toxicology and appear in numerical sequence. If a number is missing in the numerical sequence, the abstract assigned to the missing number was withdrawn by the author(s). Author names that are underlined in the author block indicate that the author is a member of the Society of Toxicology. For example, J. Smith. SOT members may sponsor abstracts that do not include an author with SOT membership. Authors who are members of designated organizations could serve as the sponsor of the abstract if an SOT member was not a co-author; these types of sponsorships are displayed with an organization name after the sponsor name (e.g., Sponsor: A. Smith, EUROTOX).

*Scientific Session Types:*

- CE Continuing Education Courses
- EC Education-Career Development Sessions
- IS Informational Sessions
- PL Platform Sessions
- PS Poster Sessions
- RI Regional Interest Sessions
- R Roundtable Sessions
- S Symposium Sessions
- W Workshop Sessions

The 2020 SOT Event App and Online Planner

The Event App is available via the SOT Annual Meeting website and app marketplaces. The Event App, alongside the Online Planner available on the SOT Annual Meeting website, enables attendees to engage with organizers, exhibitors, and each other and to manage their time and maximize their experience during the Annual Meeting. ePosters also can be accessed electronically via the Event App until May 15, 2020.

To cite a 2020 SOT Annual Meeting abstract, please format as follows: *The Toxicologist*, Supplement to *Toxicological Sciences*, 174 (1), abstract #__, 2020, Title, First Author.

Society of Toxicology
11190 Sunrise Valley Drive, Suite 300, Reston, VA 20191

www.toxicology.org