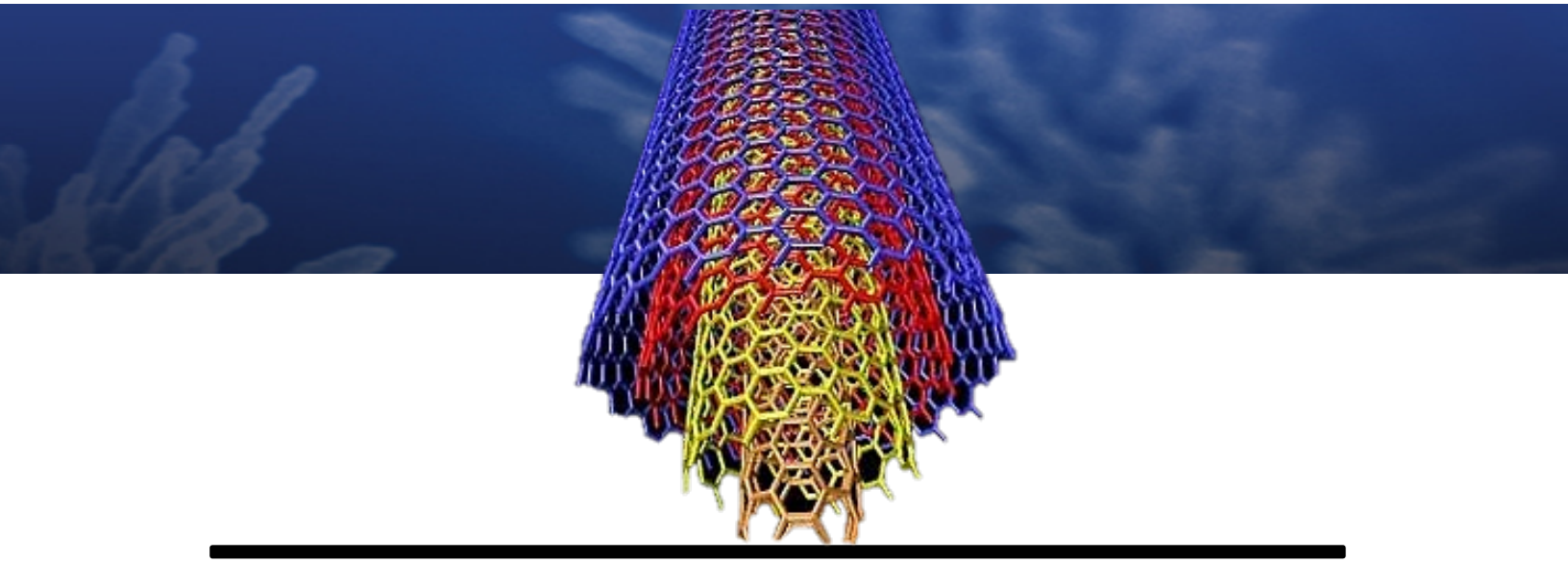
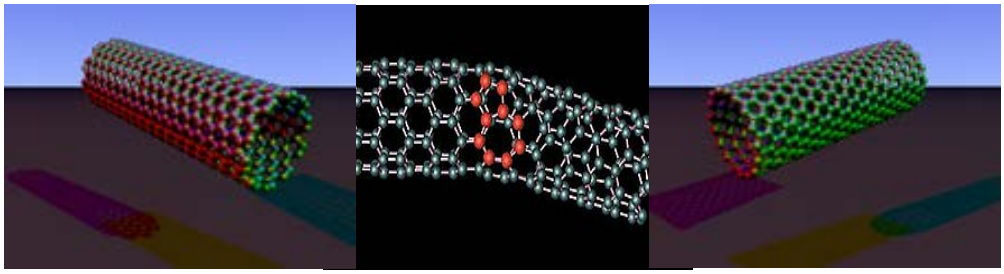


8TH INTERNATIONAL NANOTOXICOLOGY CONGRESS

nanoTOX 2016

June 1-4, 2016
BOSTON, USA



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Welcome from the chairs

On behalf of the International Advisory Board and Local Organizing Committee, we are pleased to welcome you to the 2016 International Nanotoxicology Congress, and Boston, Massachusetts, USA. This conference is the 8th in a continuing series of international meetings that are broadly focused on nanotoxicology, one that began in Boston in 2006. Thus, it is fitting that we return to this beautiful and historic city for our 10th year anniversary meeting.

The objective of this conference is to bring together scientists from academia, industry, government agencies, and non-governmental organizations to present current research findings, focus their respective talents and expertise, and initiate new collaborations in an effort to ensure the safe implementation of nanotechnology. The Advisory Board has been working hard over the past two years to develop the scientific program for this conference, and includes plenary lectures, symposia, workshops, poster sessions, and dedicated time for presentations by young scientists.

We would like to specifically recognize two organizations that have provided support, without which the 2016 conference would not have been possible. An early gift from the Turkish Society of Toxicology following the 2014 conference covered initial logistical costs. In addition, we are very grateful to the Sustainable Nanotechnology Organization (SNO) for providing the administrative and legal structures and assisting the Chairs in putting on an event of this size.

Last but not least, we would like to thank our sponsors and exhibitors for their support.

We are looking forward to interacting with all of you during the Conference and wishing you all a productive and enjoyable Conference.

Alison Elder, Co-Chair
Philip Demokritou, Co-Chair

Committees

Local Organizing Committee:

Dhimiter Bello, Univ. Mass., Lowell
James Bonner, NCSU
Jared Brown, Univ. of Colorado
Erdem Coskun, NIST/Gazi Univ.
Philip Demokritou, Harvard Univ. (co-chair)
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Robert Yokel, University of Kentucky.

A welcome message from the Sustainable Nanotechnology Organization

The Sustainable Nanotechnology Organization (SNO) welcomes you to the 2016 International Nanotoxicology Congress to Boston, Massachusetts, and we are pleased to partner with this conference. We would also like to introduce you to SNO and encourage you to become members.

SNO is a non-profit, worldwide professional society that is unique in its mission of focusing on a very new technology and its relationship to sustainability. No other organization has this combination of science and values.

SNO is comprised of individuals and institutions that are engaged in:

- Research and development of sustainable nanotechnology
- Implications of nanotechnology for Environment, Health, and Safety
- Advances in nanoscience, methods, protocols and metrology
- Education and understanding of sustainable nanotechnology
- Applications of nanotechnology for sustainability

As a professional society, SNO provides forum to advance knowledge in all aspects of sustainable nanotechnology, including both applications and implications. SNO has partnered with the Royal Society of Chemistry(RSC) to offer our members publication opportunities in *Environmental Science: Nano*. In addition, RSC supports a SNO/RSC award for Emerging Investigators. The SNO annual conference is a place where the new community of sustainable nanotechnology is being formed and advanced. The conference program is built around providing excellent talks with plenty of time for interdisciplinary networking and social interactions. SNO's Newsletter (SNO Report) provides readers with an excellent platform to discuss both the benefits and risks of nanotechnology. The SNO meeting is small enough to provide meaningful discussions and a neutral ground with both applications and implications researchers. We are very much looking forward to seeing you at SNO's 5th Annual Conference in Orlando, FL from November 12-14, 2016. This year's meeting is already proving to be exciting, featuring famous speakers from government, industry, and academia. We are focusing on the future of nanotechnology, discussing "looking back on the last five years of achievements," as well as welcoming our newest members. More information can be found on our website at www.susnano.org. Please check us out on Facebook.

We hope you enjoy this conference, and we invite you to become a member of SNO and enjoy its multiple benefits including SNO Newsletters, awards, conferences, and other opportunities.

Sincerely,

Wunmi Sadik, President, SNO
Barbara Karn, Executive Director, SNO

Program at a glance

Wednesday, June 1, 2016	
12:00-5:00 p	**Registration** *Harbor Foyer*
5:00-6:30 p	**Opening Ceremony and Panel Discussion** *Harbor Ballroom* **Participants:** Lynn Bergeson, Vincent Castranova, Lisa Friedersdorf, Mary Gulumian, Kostas Kostarelos, Harald Krug, Andrew Maynard, Nancy Monteiro-Riviere, Andre Nel, Günter Oberdörster, Kai Savolainen, Roel Schins, Vicki Stone, Treye Thomas **Moderators:** Alison Elder, Philip Demokritou
6:30-8:30 p	**Opening Cocktail Reception** *Pavilion (located in the hotel gardens)*

Thursday, June 2, 2016				
7:00am – 5:00pm	**Registration** *Harbor Foyer*			
8:00am – 9:00am	**Plenary I** *Harbor Ballroom* **Speaker: Lynn Bergeson** ***"The Nanotechnology Legal and Regulatory Landscape"***			
9:00am 9:30am	**Morning Break**			
9:30am – 12:00pm	**Parallel Session 1** *Harbor Ballroom I* **Green nanomaterials: The fusion product of green chemistry/toxicology with nanotoxicology** Chairs: Ivo Iavicoli, Anna Shvedova	**Parallel Session 2** *Harbor Ballroom II* **Emerging nanomaterials: What's next?** Chairs: Valerian Kagan, Sri Nadadur	**Parallel Session 3** *Harbor Ballroom III* **Nanoparticle dosimetry:** **Too complicated to consider too important to ignore** Chairs: Philip Demokritou, Saber Hussain	
12:00pm – 1:30pm	**Lunch** (on your own)			
1:30pm - 4:30pm	**Parallel Session 4** *Harbor Ballroom I* **Alternative testing strategies, grouping, and a tiered decision-making framework for nano-EHS regulation** Chair: Andre Nel	**Parallel Session 5** *Harbor Ballroom II* **Physiological based pharmacokinetic (PBPK) modeling of nanomaterials** Chair: Jim Riviere	**Parallel Session 6** *Harbor Ballroom III* **Ramifications of nanomaterials exposures on the maternal-fetal interface and prenatal development** Chair: Tim Nurkiewicz	**Hands-on In Vitro & In Vivo Dosimetry Workshop** *Burroughs Room* Chair: Philip Demokritou (Please note that this workshop is by invitation only for those applied and registered)
4:00pm 4:30pm	**Afternoon Break**			
4:30pm 5:30pm	**Poster Viewing (Group I)** *Galleria*			
5:30pm - 7:30pm	**Young Investigators Colloquium** *Burroughs Room* Chair: Peter Gehr Organizers: Martin Clift, Craig Poland			

Friday, June 3, 2016				
7:00am - 5:00pm	**Registration** *Harbor Foyer*			
8:00am - 9:00am	**Plenary II** *Harbor Ballroom* **Speaker: Omid Farokhzad** ***Biomedical Applications for Nanotechnology: From discovery to clinical trials of polymeric nanoparticles***			
9:00am - 9:30am	**Morning Break**			
9:30am - 12:00pm	**Parallel Session 7** *Harbor Ballroom I* **Nanotoxicology's impact on commercialization** Chairs: Christie Sayes, Jo Ann Shatkin	**Parallel Session 8** *Harbor Ballroom II* **Nanotechnology for neuroscience** Chairs: Basak Engin, Hari Shanker Sharma	**Parallel Session 9** *Harbor Ballroom III* **Adverse outcome pathways and systems biology approaches in nano-risk science** Chairs: Sabina Halappanavar, Bengt Fadeel	**Workshop on Nanoceria: Benefits and Risks** *Burroughs Room* Chair: Robert Yokel
12:00pm 1:30pm	**Lunch** (on your own)			
1:30pm - 4:00pm	**Parallel Session 10** *Harbor Ballroom I* **Standardization for nanomaterial genotoxicity testing for regulatory impact** Chairs: Erdem Coskun, Bryant Nelson	**Parallel Session 11** *Harbor Ballroom II* **Impact of the biomolecular corona on nanoparticle uptake and cellular response** Chairs: Jared Brown, Nancy Monteiro-Riviere	**Parallel Session 12** *Harbor Ballroom III* **Environmental exposures to nanomaterials: Methods, approaches, detection, and modeling** Chairs: Teresa Fernandes, Jamie Lead, Navid Saleh	**Nanoceria Workshop** *Burroughs Room* Chair: Robert Yokel
4:00pm - 4:30pm	**Afternoon Break**			
4:30pm - 6:00pm	**Poster Viewing Group II** *Galleria*			
6:00pm - 7:00pm	**Break**			
7:00pm - 10:00pm	**Conference Dinner and Awards Ceremony** *Harbor Ballroom* Hosts: Alison Elder, Philip Demokritou			

Saturday, June 4, 2016			
7:00am – 12:00pm	**Registration** *Harbor Foyer*		
8:00am – 9:00am	**Plenary III** *Harbor Ballroom* **Speaker: Paul Weiss** ***Nanoscience Approaches to Heterogeneity in Biological Systems***		
9:00am - 9:30am	Morning Break		
9:30am - 12:00 pm	**Parallel Session 13** *Harbor Ballroom I* **Lessons from the real world: What is human toxicology telling us?** Chairs: Dhimiter Bello, Alison Elder	**Parallel Session 14** *Harbor Ballroom II* **Nanomaterials in food and agriculture** Chair: Hongda Chen, Philip Demokritou	**Parallel Session 15** *Harbor Ballroom III* **Lessons from mouse models of susceptibility to ENM-induced lung disease** Chairs: Jamie Bonner, Andrij Holian

Detailed daily program

Wednesday June 1, 2016

5:00pm – 6:30pm **Opening Plenary**, Harbor Ballroom

Welcoming Remarks from Chairs: Philip Demokritou, Alison Elder

Panel Discussion:

Lynn Bergeson, Vincent Castranova, Lisa Friedersdorf, Mary Gulumian, Kostas Kostarelos, Harald Krug, Andrew Maynard, Nancy Monteiro-Riviere, Andre Nel, Günter Oberdörster, Kai Savolainen, Roel Schins, Vicki Stone, Treye Thomas

6:30pm – 8:30pm **Cocktail reception,** The Pavilion

Thursday, June 2, 2016

8:00am – 9:00am Plenary I:

The nanotechnology legal and regulatory landscape

Speaker: Lynn L. Bergeson
Bergeson & Campbell, P.C.

Description: The inclusion of manufactured nanomaterials in manufacturing operations and finished products is common today. While the pace of technological innovation is rapid, the unambiguous application of law, regulation, and policy, and the implications of nanotechnology for effective product stewardship are moving at lesser speeds, creating some measure of uncertainty and potential commercial, legal, and business risk. It is, for example, unclear what standards apply in all cases to various phases of a product's development, use, and end of life if the product contains nanoscale materials and are expected to remain in the product after its useful life. While emerging regulatory, legal, and policy initiatives are lessening the uncertainty, the application of legally enforceable standards is sometimes fluid, leaving product manufacturers and other nano stakeholders in a quandary.
This presentation will focus on the emerging legal and regulatory developments pertinent to nanotechnology. le the focus will favor U.S. law and policy, foreign legal programs will be included because some are particularly influential, including the European Union's (EU) Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) program and a growing number of notification requirements derivative of European "nanomaterial product inventories."

Biosketch: Lynn L. Bergeson; Owner of Bergeson & Campbell, P.C. (B&C®), Ms. Bergeson has earned an international reputation for her deep and expansive understanding of the Toxic Substances Control Act (TSCA), the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), European Union Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), and especially how these regulatory programs pertain to nanotechnology, biotechnology, and other emerging transformative technologies. Her knowledge of and involvement in the policy process allows her to develop client focused strategies whether advocating before Congress, the U.S. Environmental Protection Agency (EPA), the U.S. Food and Drug Administration (FDA), or other governance and standard-setting bodies.

Ms. Bergeson counsels corporations, trade associations, and business consortia on a wide range of issues pertaining to chemical hazard, exposure and risk assessment, risk communication, minimizing legal liability, and evolving regulatory and policy matters pertinent to products of conventional, biotechnology, biobased chemicals, nanotechnologies, and other emerging technologies, particularly with respect to TSCA, FIFRA, Food Quality Protection Act (FQPA), REACH and REACH-like programs, and Occupational Safety and Health Administration (OSHA) matters.

Parallel Sessions 1 through 6

Parallel session 1 (9:30 – 12:00pm, Harbor Ballroom I)

Green nanomaterials: The fusion product of green chemistry/toxicology with nanotoxicology
Chairs: Anna A. Shvedova, NIOSH/WVU, USA and Ivo Iavicoli, University of Naples Federico II, Italy

Description: Integration of nanotoxicology with green chemistry and green toxicology is the basis for the design and development of green nanomaterials. This will form a new area of science encompassing chemicals and
processes powering to reduce negative impacts of nanomaterials on human health and the environment resulting in enhanced sustainability and safety. The major objective of "green" nanotoxicology is based on the necessity of developing novel "green" nanomaterials that overcome their intrinsic theoretical bulk limits by changing the fundamental underlying physics, chemistry while keeping the manufacturing cost along with footprint of adverse health/environmental outcomes to a minimum. USA, Europe and the OECD countries are moving towards regulatory strategies encouraging sustainable, safe and green nanotechnology based on advanced research discovering the ecology-friendly nanomaterials and their applications. The objective of this session is to bring together scientific experts from academia, industry, and government agencies from around the world to present and discuss current research findings on the subject of green nanotechnology, nanotoxicology, sustainability and safety.

Program:

9:30am	Greenness and Sustainability: Joel A. Tickner, University of Massachusetts, Lowell, USA
9:55am	Opportunities and challenges of nanotoxicology in the green economy: Ivo Iavicoli, University of Naples Federico II, Italy
10:20am	Nanocellulose green natural products: toxicology prospective: Anna. A. Shvedova, NIOSH and WVU, USA
10:45am	Nano-pesticides: Environmental Fate and Exposure Modeling: Melanie Kah, Department of Environmental Geosciences, University of Vienna, Austria
11:10am	Green Toxicology meets Nanotoxicology: The Process of Sustainable Nanomaterial Development and Use: Harald Krug, Empa, Switzerland
11:35pm	Occupational safety and health: green chemistry, sustainability and regulatory policy: Paul A. Schulte, CDC/NIOSH, USA

Parallel session 2 (9:30am – 12:00pm, Harbor Ballroom II)

Emerging nanomaterials: What's next?
Chair: Sri Nadadur, NIEHS, USA

Description: Technological advancements in material science promise ever increasing number of engineered nanomaterials. This list of novel materials is not only increased in dimensionality, but also includes diverse noble transition materials, whose behavior in biological matrix is not known. Complemented with diverse biopolymers, like DNA, the new nanomaterials engage in unpredictable interactions leading to non-linear bio-responses. This session will provide an overview on the state of these emerging novel materials to educate the Nanotoxicology field for expert assessments of their safety as a requirement for the effective and broad technical and biomedical applications.

Program:

9:30am	Introduction – Sri Nadadur, NIEHS, USA
9.35am	Environmental and Health Effects of Emerging Two-dimensional Materials Apparao M. Rao, Clemson University, USA
10.05am	Emerging Biosensing Applications of Carbon Nanotubes and Graphene with Implications for Mitigating Nanotoxicology by Design Michael S. Strano, Massachusetts Institute of Technology, USA
10.35am	Overcoming the Endosomal Escape Problem: Lysosome-Targeting Gold Nanostar Nanoconstructs Terri W. Odom, Northwestern University, USA
11.05am	A Nanoengineers's Perspective on EHS Topics in Emerging Advanced Nanoengineered Hierarchical Materials Brian Wardle, Massachusetts Institute of Technology, USA
11.35am	Fine-tuning properties of carbon nanomaterials for biomedical applications Alexander Star, University of Pittsburgh, USA

Parallel session 3 (9:30am – 12:00pm, Harbor Ballroom III)

In-vitro and In-vivo dosimetry of engineered nanomaterials: Too complicated to consider too important to ignore.
Session Chairs: Philip Demokritou, PhD Harvard School of Public Health, USA, and Saber Hussain, Wright Patterson Air Force Base, USA

Description: Due to the potential public health risk arising from exposure to engineered nanomaterials (ENMs) through consumer applications, a thorough evaluation of their safety is essential. Owing to the fast pace of ENM generation, high-throughput in vitro methods for safety assessments are sorely needed, but to date have proven unreliable with limited predictive capabilities extending to in vivo models. One major contributor to the discrepancies that exist between these models is a failure to reconcile in vitro and in-vivo dosages. Despite growing evidence of the importance of ENM dosimetry for accurate hazard assessments, few toxicological studies take it into consideration. This oversight is likely due to a lack of standardized, easy to use, and validated methodologies for dispersion preparation, characterization and in vitro dosimetry estimation.

This session will highlight recent advancements that strengthen our understanding on ENM in-vitro and in-vivo dosimetry, including the development of aerosol lung deposition models, generation of pertinent in vitro exposure systems using air liquid interface systems, and integrated approaches for calculating and predicting relevant dosages for both in vitro and in vivo nanotoxicological studies. It will also highlight several mature, sophisticated computational tools and experimental methods for obtaining dosimetry information in vivo and extrapolating those findings to in vitro cellular systems with specific examples related to “real world” ENM exposures. In addition to discussing the current state of the art regarding ENM dosimetry, discussions will center on the need and means for future development of this area.

Program:

9:30am	Introduction to the session and its objectives Saber Hussain, WPAFB, USA
9:35am	Emerging tools and approaches for in vitro dosimetry of engineered nanomaterials, Philip Demokritou, Harvard School of Public Health, USA
10:05am	Advanced Computational fate and transport modeling for In Vitro Nanomaterial Dosimetry, Glen Deloid, Harvard School of Public Health, USA
10:35am	Engineered Nanomaterial deposition in the respiratory tracts of humans and animals, Bahman Asgharian, ARA, Inc., USA
11:05am	Nanoparticle dosimetry to the point - Air-liquid exposure systems to deliver an accurate dose onto the lung cell surface in vitro, Barbara Rothen-Rutishauser, University of Fribourg, Switzerland
11:35am	In Vitro Aerosol Exposure Systems: Challenges in Dosimetry and Strategic Solution, Trevor Tilly, Air Force Research Laboratories, USA

Parallel session 4 (1:30pm – 4:30pm, Harbor Ballroom I)

Alternative test strategies, categorization and a tiered decision-making framework for nano EHS Regulation
Chair: André Nel, UCLA, USA

Description: Predictive toxicology includes a broad range of methods that can generate useful information useful nano EHS decision analysis. Predictive toxicology focuses upon mechanisms of toxicity or adverse outcome pathways (AOP), which can be used to forecast adverse *in vivo* events based on the contribution of the AOP to the pathophysiology of disease. Increasing reliance on *in vitro* testing as envisioned by reports by the National Academy of Sciences and by European authorities is being considered as a means for limiting the amount of animal studies and cost. ENMs of interest are introduced into the test system, and observations are made regarding biological perturbations, allowing researchers to evaluate whether the materials being tested are implicated in the initiation or progression of an AOP. The value of mechanistically-based *in vitro* assays can be vastly expanded through the use of high throughput screening (HTS). HTS allows researchers to simultaneously test a large number of materials, which can be ranked and the results used for *in vivo* testing that limit the number of animals used, and provide readouts in which the AOPs is reflected in the pathophysiology of disease. These *in vitro/in vivo* comparisons can be used to develop a tiered risk analysis framework, which together with exposure analysis and nanomaterial categorization can be included in decision analysis. The symposium will discuss the utility and shortcomings of alternative test strategies, predictive toxicological approaches and tiered decision analysis, including assessment of nanomaterial hazard in the environment.

Program:

1:30pm	Use of alternative test strategies and AOP-based high content screening for predictive hazard profiling and tiered risk assessment André Nel, UCLA, USA
2:10pm	A decision-making framework for testing and grouping of nanomaterials Robert Landsiedel, BASF, Germany
2:40pm	Use of predictive approaches in EHS regulation Jeff Morris, US Environmental Protection Agency, USA
3:00pm	Implementing environmentally-relevant exposures and alternative test strategies to assess environmental nanotoxicology Patricia Holden, UCSB, USA
3:30pm	Alternative toxicity testing of nanomaterials using 3D human lung microtissues Agnes Kane, Brown University, USA
3:45pm	Discussion panel with questions from the audience

Parallel session 5 (1:30pm – 4:30pm, Harbor Ballroom II)

Physiological based pharmacokinetic (PBPK) modeling of nanomaterials
Chair: Jim Riviere, Kansas State University, USA

Description: This symposium will introduce and overview the progress of utilizing PBPK modeling techniques as applied to the unique issues surrounding nanomaterials. Questions including "Why are they needed?" and "What unique advantages do they provide to nanotoxicology?" will be addressed. The utility of applying PBPK as a tool to link in vitro to in vivo studies, as well as facilitate interspecies extrapolations will be discussed. The existing models will be thoroughly reviewed by all speakers. In addition, limitations of in vivo studies will be discussed and directions of what needs to be done in the future outlined.

Program:

1:30pm	Physiological Based Pharmacokinetic (PBPK) Modeling of Nanomaterials: Why is Quantitation and Anatomical / Physiological Reality So Important? Jim Riviere, Institute of Computational Comparative Medicine, Kansas State University, USA
1:50pm	Using Toxicokinetic Modeling to Describe and Predict the Fate of Inorganic Nanoparticles in the Body Gerald Bachler, ETH Zurich, Institute for Chemical and Bioengineering, Zurich, Switzerland and Shell International, The Hague, Netherlands
2:20pm	PBPK Modeling of Gold Nanoparticles: A Tool to Extrapolate from Animals to Man (30 mins) Zhoumeng Lin, Institute of Computational Comparative Medicine, Kansas State University, USA
2:50pm	PBPK Modeling of Nanopharmaceuticals: Model structures and Limitations Imposed by Idiosyncratic Infusion-Related Reactions S. Moein Moghimi, Centre for Pharmaceutical Nanotechnology and Nanotoxicology, University of Copenhagan, Denmark.
3:20pm	Contrasting the in vivo Processing Variances of Nanoceria in Organs: Major Differences between Spleen and Liver Revealed Uschi Grahm, University of Kentucky Center for Applied Energy Research, USA
3:40pm	How does the Thickness of 2D Graphene Oxide Sheets determine their Biological Fate and in vivo Dosimetry? Dhifaf Jasim, University of Manchester, UK

Parallel session 6 (1:30pm – 4:30pm, Harbor Ballroom III)

Ramifications of nanomaterial exposures on the maternal-fetal interface and prenatal development
Chairs: Timothy R. Nurkiewicz, West Virginia University, USA

Description: The impact of nanomaterials on various biological systems has become obvious in recent years. The details of such exposures have also been characterized to various extents, in terms of: physicochemical properties, dose-response and mechanisms. Unfortunately, the reproductive system is one of the most poorly studied systems in the field of nanotoxicology. This symposium will identify how nanomaterial exposures during pregnancy can influence fetal outcomes. The potential/risk for exposure and consequence will be discussed at the maternal, placental and fetal levels. The impact of such nanomaterial exposures on fetal genetics, specific organ end points, as well as the foundation for the development of adult disease will be the broader theme of the symposia. The greater goal of the symposia is to lay the foundation and future directions of critically needed maternal-fetal nanotoxicology research.

Program:

1:30pm	Opening comments, symposia overview/purpose/goals. Tim Nurkiewicz, West Virginia University School of Medicine, USA
1:40pm	Nanoparticle transport across the placental barrier: Pushing the field forward! Peter Wick, Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland
2:05pm	Uterine and Fetal Microvascular Consequences of Nanomaterial Exposures. Phoebe Stapleton, West Virginia University School of Medicine, USA
2:30pm	Prenatal Nephrotoxicity and Maternal Nanomaterial Inhalation. Jason Blum, New York University Medical Center, USA
2:55pm	Prenatal exposure to nanomaterials and effects on somatic DNA in the offspring and the germline Ulla Vogel, National Research Centre for the Working Environment, Denmark
3:20pm	Nanoparticle Coating and Genetic Make-Up of the Host Modulate Genotoxicity of Ingested Silver Nanoparticles. Ramune Reliene, University at Albany, State University of New York, USA
3:45pm	General discussion

Dosimetry Workshop

1:30pm – 5:30pm, Burroughs Room

(This is an event by invitation only, pre-registration is required)

Assessing the dose of nanomaterials in toxicological studies: Advanced approaches utilizing experimentation and fate and transport modeling

Chairs: Philip Demokritou, Harvard University, USA; Flemming Cassee, RIVM Netherlands
Instructors: Bahman Asgharian (ARA, USA), Sandra V. Pirela (Harvard University), Glen DeLoid (Harvard University) and Joel Cohen (Harvard University)

Objective:
The objective of this workshop is to review the tools currently available to nanotoxicologists for quantifying particle dosimetry in both in vitro and in vivo experimental models. This includes a series of brief lectures that will provide the necessary background information/concepts and overview of methods in addition to hands-on training of both experimental and computational approaches for in vitro/in vivo dosimetry. Participants will be trained using a number of relevant real world case studies that demonstrate the applications of the emerging methods presented here.

Introduction:
In vitro high-throughput screening platforms based on mechanistic injury pathways have been used for hazard assessment of engineered nanomaterials (ENMs). Toxicity screening and other in vitro nanotoxicology assessment efforts typically compare and rank the bioactivity of nanomaterials relative to each other. It has been shown that ENMs hazard rankings are highly sensitive to variability in poorly standardized dispersion protocols and lack of dosimetry. This sensitivity is largely due to the impact of particle transformations on partico-kinetics that affect bioactivity and delivery of particles to cells. The importance of such dosimetric considerations apply to studies using both cell cultures as well as animal experimental models for toxicology assessments. Thus, extrapolation to humans becomes more reliable once the dose is determined accurately at the site of deposition.
Emerging hybrid, experimental/computational approaches to cellular dosimetry can be used by nanoparticle toxicologists to accurately calculate the delivered to cell dose metrics for various ENMs and in vitro experimental conditions as a function of exposure time. In addition, in vivo lung deposition models allow researchers to estimate the delivered particle dose in any region of the respiratory system, as well as study the implications of particle properties and breathing parameters. More importantly, such dosimetric methodologies enable nanoparticle toxicologists to bring in vitro and in vivo doses to the same scale, an important step towards the development and validation of in vitro cellular screening assays.

Program:

1:30pm Objectives and opening remarks about the Workshop An overview of emerging tools for the in vitro and in vivo dosimetry of engineered nanomaterials (Philip Demokritou)

1:40pm Case studies using the Multple-Path Particle Dosimetry (MPPD) model (Bahman Asgharian)
Description of MPPD model

Hands on training with MPPD model using data set for engineered nanoparticles to determine mass flux in the lung and deposited dose metrics

Both human and rodent dosimetry parameters will be used

In Vitro Dosimetry - Part A: Overview and description of the Harvard integrated in vitro dosimetry platform

Program:

2:30pm Overview of the Harvard Integrated In Vitro Dosimetry platform (Sandra Pirela, Glen DeLoid, Joel Cohen)
Introduction to the Harvard Integrated In Vitro Dosimetry Platform
Before getting started:
Calorimetric calibration of your sonicator
Measurement of DSEcritical for ENM
Detailed description of all of the steps in the protocol
Step 1: Suspension preparation
Step 2: Particle Suspension characterization
Step 3: DG in vitro fate and transport modeling

3:40pm Break

In vitro dosimetry - Part B: Case study

4:00pm Using the Harvard Integrated In Vitro Dosimetry platform for the case study of nano- CeO2 (Sandra Pirela, Glen DeLoid, Joel Cohen) Preparation of the ENM suspension in water and in media (Step 1)
Characterization of the prepared suspension (Step 2)
Size of formed agglomerates - using Dynamic Light Scattering (DLS)
Measurement of effective density using Harvard Volumetric Centrifugation method (VCM)
Perform Fate and Transport modeling for dosimetry using Harvard DG model –MATLAB is required (Step 3)
Dosimetry data analysis

5:20pm Questions and session wrap-up

Young investigators colloquium

Chair: **Prof. Em. Peter Gehr (University of Bern, Switzerland)**

Keynote Speaker: **Dr. Roel P. F. Schins (IUF, Duesseldorf, Germany)**

Outlook of Session: The space available for this session is limited, so priority will be given to selected presenters and their colleagues. It is considered to be quasi-informal, in that there are still formal scientific presentations, but there will be drinks and snacks available (to all). In addition, this is to be a 'safe' environment for young investigators to present their research to their peers and the hierarchy of the field.

Timetable for Session

5:30hrs		**Introduction to Session by Chair**
5:35hrs		**Keynote Presentation**
Dr Roel Schins		Title: TBD
6:00 – 7:20pm		**Young Investigator Presentations**
1	Sandra Pirela	Engineered Nanoparticles Emitted from Laser Printers: A Case Study Of Environmental Health Implications From Nano-Enabled Products During Consumer Use
2	Savvina Chortarea	Biological impact of sub-chronic repeated exposures to aerosolised MWCNTs on healthy and asthmatic lung cells at occupational relevant doses
3	Sarah Søs Poulsen	Multi-walled carbon nanotube physicochemical properties predict pulmonary inflammation and genotoxicity
4	Dhifaf Jasim	How does the Thickness of 2D Graphene Oxide Sheets determine their Biological Fate and in vivo Dosimetry?
5	Julia Kolling	Toxicological assessment of shape-engineered titanium dioxide nanoparticles
6	Laura Rubio Lorente	Cerium Oxide nanoparticles moderately enhances the cancer features exhibited by cigarette smoke condensate in a human lung epithelial cell line
7	Valerie Minarchick	Phenotypic Differences in Aortic and Microvascular Endothelial Cells Influences Responses to Cerium Dioxide Nanoparticles
8	Marcella De Maglie	Repeated oral administration of low doses of silver in mice: Effects on central nervous system
7:20pm		**Closing of Session by Chair**
7:30pm (5-10mins)		**Meeting of Judging Panel**

Young Investigator: A young investigator is defined as one within the final year of their PhD, and not beyond 3 years of their post-doctoral studies.

Presentations: Presentations will be 10mins in total, with 7mins allowed for presentation as well as 3 minutes for discussion. Those giving the presentation will be determined from the abstract submission stage. On the abstract submission form, there will be an option for people interested in presenting at the session to complete. The abstracts will then be reviewed by a select panel to determine their applicability for the session itself. Presenters will give a biography of themselves prior.

Prizes: First Prize for 'best young investigator presentation' of $500 and runner up prize of $200prize. The winner will be determined by a select panel of senior scientists basing their decision upon a specific set of criteria (*i.e.* scientific content, presentation style, ability to answer questions).

Abstract Panel: Dr. Craig A. Poland and Dr. Martin J. D. Clift

Grouping Rationale: The presentations cross an array of different materials considering incidental exposures such as from printer emissions and occupational exposures to biomedical applications (GO). The first 4 presentations are grouped loosely around carbonaceous nanomaterials with talks 2-4 considering high aspect ratio nanoparticles. The shape aspect forms a link to talk 5 which considers the impact of shape on TiO2 toxicity. This talk loosely groups together with talks 6 and 7 and these three talks (5-7) all consider metal oxides; specifically, TiO2 and CeO4. The final talk considers metal (Ag) toxicity on the central nervous system.

Friday, June 3, 2016

8:00am – 9:00am Plenary II:

Biomedical applications for nanotechnology: from discovery to clinical trials of polymeric nanoparticles

Speaker: Omid C. Farokhzad
Laboratory of Nanomedicine and Biomaterials, Department of Anesthesiology
Brigham and Women's Hospital and Harvard Medical School

Description: Controlled-release polymeric nanoparticles can deliver drugs in the optimum dosage over time, thus increasing the efficacy of the drug, maximizing patient compliance and enhancing the ability to use highly toxic, poorly soluble, or relatively unstable drugs, and can also be used to co-deliver two or more drugs for synergistic combination therapy. Moreover, the surface engineering of these nanoparticles may yield them "stealth" to prolong their residence in blood, and the functionalization of these particles with targeting ligands can differentially target their delivery or uptake by a subset of cells, further increasing their specificity and efficacy. Nevertheless, the successful clinical translation of targeted polymeric nanoparticles for drug delivery requires optimization of many distinct parameters including: variation in the composition of the carrier system, drug loading efficiency and release kinetics, surface hydrophilicity, surface charge, particle size, density of possible ligands for targeting, etc., resulting in potential variables for optimization which is impractical to achieve using a low throughput approach. Combinatorial approaches precisely engineer nanoparticles and screen multiple nanoparticle characteristics simultaneously with the goal of identifying formulations with the desired physical and biochemical properties for each specific application. In this talk, I will present our efforts in the design and optimization of targeted polymeric nanoparticles for medical applications, which formed the foundation for the clinical translation of the first in-human targeted and controlled-release nanoparticles.

Biosketch: Omid Farokhzad is an Associate Professor at Harvard Medical School (HMS) and a physician-scientist in the Department of Anesthesiology at Brigham and Women's Hospital (BWH). Dr. Farokhzad directs the Laboratory of Nanomedicine and Biomaterials at BWH. He is a faculty member of the Brigham Research Institute Cancer Research Center. He is additionally a member of the Dana Farber/Harvard Cancer Center Programs in Prostate Cancer and Cancer Cell Biology. Dr. Farokhzad's research is focused on the development of therapeutic nanoparticle technologies; most notably, he pioneered the high throughput combinatorial development and screening of multifunctional nanoparticles for medical applications. Dr. Farokhzad has authored approximately 130 papers (~24,000 citations; H-Index 62) and holds more than 145 issued/pending US and International patents. The technologies that Dr. Farokhzad has developed with collaborators at HMS and MIT formed the basis for the launch of four biotechnology companies: BIND Therapeutics (NASDAQ: BIND), Selecta Biosciences, Tarveda Therapeutics (formerly Blend Therapeutics), and Koan Biotherapeutics, which are translating the aforementioned academic innovations toward commercialization and societal impact. Dr. Farokhzad has served in various capacities on the Board of Directors and the Scientific Advisory Board of these companies. He was a recipient of the 2013 RUSNANOPRIZE, one of the largest international nanotechnology prizes, for the development and industrialization of nanoparticle technologies for medical applications. In 2014, he received the Golden Door Award from the International Institute of New England for his societal and economic impact as a naturalized USA citizen. In 2015, he was named as one of The

Worldview 100 by Scientific American, which recognized visionaries who shape biotechnology around the world. In 2016, he was among the recipients of the Ellis Island Medal of Honor for his scientific, societal and economic contributions to America as an immigrant. Dr. Farokhzad was elected to the College of the Fellows of the American Institute of Medical and biological Engineering. He was selected by Thomson Reuters among the Highly Cited Researchers in 2014 and 2015. The Boston Globe selected him among the top innovators in Massachusetts and the Boston Business Journal selected him among the Health Care Champions for his innovations. In 2012, he was among the regional Ernst & Young Entrepreneur of the Year awardees. Dr. Farokhzad completed his post-graduate clinical and post-doctoral research trainings, respectively, at the BWH/HMS and MIT in the laboratory of Institute Professor Robert Langer. He received his M.D. and M.A. from Boston University School of Medicine and his M.B.A. from the MIT Sloan School of Management.

Parallel Sessions 7 through 12

Parallel Session 7 (9:30am – 12:00pm, Harbor Ballroom I)

Nanotoxicology's Impact on Commercialization
Session Co-chairs: Jo Anne Shatkin, Vireo Advisors, LLC, USA and Christie Sayes, Baylor University, USA

Provocative Questions:
Have nanotoxicology findings handicapped commercialization?
Or has new thinking in health & safety research strengthened the product value chain?

Description: The development of the field of nanotoxicology has emerged quickly, while companies are advancing commercial development of novel materials and products. How has this rapid growth affected companies and their success? This session includes presentations and interactive discussion about the impacts of nanotoxicology on commercialization efforts by companies. Following an introduction, we will hear perspectives from four industry representatives about their experiences in obtaining and using toxicology information, or impacts from published studies on legacy products in diverse applications and markets. The remainder of the session will be a moderated roundtable interactive discussion with speakers about key past developments, ongoing issues, and challenges for the future.

Program:

9:30am	Introduction of Topic and Speakers. Jo Anne Shatkin
9:45am	An EH&S Approach for Commercialization of Novel Forms of Nanocellulose Kimberly Nelson, American Process, Inc., USA
10:10am	Use of Life Cycle Analysis in Responsible Design of a Quantum Dot Display Product and Manufacturing Process. Robert Nick, QD Vision, USA
10:45am	Nanotoxicology's Impact on a Conventional Material Producer. Shaun Clancy, Evonik, USA
11:10am	Searching for Risk, Finding Value: Unexpected Developments from Nanotoxicology/Industry Collaborations. Steven Oldenberg, nanoComposix, USA
11:35am	Roundtable and Q&A, with audience participation
11:55am	Wrap-up

Parallel Session 8 (9:30am – 12:00pm, Harbor Ballroom II)

Nanotechnology for neuroscience
Chairs: Ayse Basak ENGIN, Gazi University, Turkey and Hari Shanker Sharma, Uppsala University, Sweden.

Description: Neurological disorders affect hundreds of millions people worldwide. It is estimated that there are globally 35.6 million people with dementia with 7.7 million new cases every year, while more than 50 million people have epilepsy. Despite enormous advances in brain research, central nervous system disorders remain the world's leading cause of disability in part due to the inability of the majority of drugs to reach the brain parenchyma. Therefore, nanotechnology and nano-sized materials might be a good tool to overcome the deficiencies of the available therapeutic strategies. In this session, blood brain barrier (BBB), its major limitations for drug delivery to the brain and targeted controlled delivery across the BBB using a multifunctional nanoconstruct will be presented. Afterwards, the neuroprotection as well as the neurotoxicity of nanoparticles in the central nervous system with special reference to nanomedicine and the latest developments and the experimental investigations on nanodrug delivery in several models of neurotrauma in achieving neuroprotection will be discussed in detail. Later, in neurodegenerative disorders, as the causes of dopaminergic neuronal damage still remain unknown, for nanostructured drugs, the impact of identification of more specific targets such as N-methyl D-aspartate receptors will be presented. Lastly, long-term effects and biodegradation kinetics of chemically functionalized carbon nanotubes as promising nanovectors for brain applications within primary microglia will be discussed. Thus this session will critically evaluate the recent strategies and developments in targeting the nanodrugs to brain, as well as the application and impact of nanotechnology in neurological disorders.

Program:

9:30am	Neuroprotection and neurotoxicity of Nanoparticles in the Central nervous system with special reference to nanomedicine Hari Shanker Sharma, Uppsala University, Sweden
10:05am	Targeting nanoparticles to brain: Impact of N-methyl-D-aspartate receptors Ayse Basak Engin, Gazi University, Turkey
10:40am	Long-term impact and biodegradation kinetics of chemically functionalized carbon nanotubes within primary microglia Cyrill Bussy, University of Manchester, UK
11:15pm	Effects of SiO_2 and CeO_2 nanoparticles on Alzheimer-like pathology in mice after 3 and 14 weeks' oral exposure Roel P.F. Schins, Germany
11:50am	Conclusion: Complex interaction of microglia, astrocyte and neuron in nanoparticle exposed brain Discussion by all participants

Parallel session 9 (9:30am – 12:00pm, Harbor Ballroom III)

Adverse outcome pathways and systems biology approaches in nano-risk science
Chairs: Sabina Halappanavar, Health Canada, Ottawa, Canada and
Bengt Fadeel, Karolinska Institute, Sweden

Description: Toxicological assessment is moving away from the traditional endpoint-based model to gaining a better understanding of more organized, systems-level responses following exposure that have predictive value for risk assessment purposes. This session will focus on ways in which such information can be exploited to understand mechanisms of response to nanomaterials using specific examples from well-studied materials, to explore responses that can be used as biomarkers of exposure, and how to build predictive frameworks based on broad-based data sets.

Program:

9:30 am	Introductory remarks. Bengt Fadeel, Karolinska Institute, Sweden
9:35 am	Adverse outcome pathways: A framework for organizing mechanistic information to improve toxicity assessment. Kristie Sullivan, Physicians for Responsible Medicine, USA
9:55 am	Advances in developing adverse outcome pathways to assess inhalation toxicity of multi-wall carbon nanotubes Sybille van den Brule, Université Catholique de Louvain, Belgium
10:15 am	Omics, bioinformatics, and adverse outcome pathways in nano safety research. Sabina Halappanavar, Health Canada, Canada
10:35 am	Global gene expression profiling in nanosafety for hazard identification, grouping, and ranking and risk assessment Ulla Vogel, National Research Centre for the Working Environment, Denmark
10:55 am	Endoplasmic reticulum stress is an earlier biomarker for nanotoxicological evaluation Chunying Chen, National Center for Nanoscience and Technology, China
11:10 am	A combined proteomics and metabolomics approach to assess the effects of gold nanoparticles in vitro Sabrina Gioria, JRC, Italy
11:22 am	Short-term inhalation exposure to copper oxide nanoparticles induces gene expression changes associated with inflammation and cell proliferation in the rat bronchoalveolar epithelium. Pedro M. Costa, Karolinska Institute, Sweden
11:34 am	Nanoparticle exposure of persistently herpesvirus-infected lung cells reactivates latent virus and restores features of an acute virus infection in vitro and in vivo Tobias Stöger, Helmholtz Zentrum, Munich, Germany
11:46 am	General discussion and acknowledgements Sabina Halappanavar

Parallel session 10 (1:30pm – 4:00pm, Harbor Ballroom I)

Standardization for Nanomaterial Genotoxicity Testing for Regulatory Impact
Chairs: Bryant C. Nelson and Erdem Coskun, NIST, USA, Shareen H. Doak, Swansea University, UK and Maria Dusinska, Norweigian Institute for Air Research, Norway

Description: Many of the current investigations on the environmental and human health risks of engineered nanomaterials focus on their short-term acute toxicity. However, the long-term chronic effects of nanomaterials on living systems, and in particular, on the genome of living systems, also warrant attention. An increasing number of nanomaterial safety studies include an assessment of genotoxicity as part of the overall risk evaluation. Nanomaterials have the potential to directly interact with DNA, to indirectly modify components of the genetic machinery and/or to induce inflammation and promote the *in vivo* formation of reactive oxygen species in organisms and humans. Nanomaterial induced genotoxicity can result from a single process or more likely via a combination of multiple *in vivo* processes. Subsequent modification of genomic DNA could lead to the development of mutagenesis, carcinogenesis or other chronic conditions. This symposium will emphasize recent developments in the measurements, test methodologies/models and test strategies that support robust hazard assessments. Standardization efforts, development of standards for measurements and alternative test strategies for nanomaterial genotoxicity determinations that are likely to have broad regulatory impact will also be emphasized.

Program:

1:30pm	Nanomaterial genotoxicity testing Rosalie Elepuru, FDA, USA
2:00pm	Adaptation of the OECD guidelines to support nanogenotoxicity hazard assessment Shareen H. Doak, Swansea University, UK
2:20pm	Absolute identification and quantification of DNA repair proteins: An emerging measurement trend in nanotoxicology Erdem Coskun, NIST, USA
2:40pm	Advances in 3-dimensional in vitro models as well as with in vivo approaches for genotoxicity testing of nanomaterials Stefan Pfuhler, Procter & Gamble, USA
3:10pm	Emerging metrology for high throughput nanomaterial genotoxicology Bryant C. Nelson, NIST, USA
3:30pm	Advances in our understanding of nanomaterial carcinogenicity Maria Dusinska, Norwegian Institute for Air Research, Norway
3:45pm	Sustained inflammation and genotoxicity following pulmonary exposure to graphene and graphene oxide Stefan Bengtson, National Research Center for the Working Environment, Denmark

Parallel Session 11 (1:30pm – 4:00pm, Harbor Ballroom II)

Impact of the biomolecular corona on nanoparticle uptake and cellular response
Chairs: Jared Brown, University of Colorado, USA and Nancy A. Monteiro-Riviere, Kansas State University, USA

Description: Proteins and other biomolecules are known to interact with surfaces via a variety of mechanisms. For nanoparticles, such interactions may affect size distributions, surface oxidation reactions, dissolution kinetics, and the manner in which the particles interaction with cells. Likewise, the physicochemical properties of the nanoparticles themselves can affect interactions of the surface with biomolecules. Such process are likely to influence nanoparticle biodistribution and clearance, as well as cell- and tissue-level toxicological responses. This session will focus on the state-of-the-art regarding knowledge of nanoparticle-protein interactions and evidence from fundamental research that demonstrates an impact on biological responses.

Program:

1:30pm	The biocorona: Lessons learned and challenges accepted Roland Stauber, ENT Medical University Mainz, Germany
1:55pm	Role of physicochemical properties of nanoparticles on biocorona formation and cellular uptake profiles in endothelial cells Parwathy Chandran, Kansas State University, NICKS Center, USA
2:20pm	Disease-induced modification in the identity of the nanoparticle-biocorona and toxicity. Jonathan Shannahan, Purdue University, USA
2:45pm	Influence of nanoparticle surface chemistry on agglomeration and lung lining/lipid/protein interaction. Andrea Haase, German Federal Institute of Risk Assessment, Germany
3:10pm	Silica coating influences the corona and biokinetics of cerium oxide nanoparticles. Nagarjun Konduru, Harvard University, USA
3:35pm	Proteomics identification of in vivo serum and plasma protein adsorbed to nanoceria: Insights into the potential pathways by which cerium oxide nanoparticles may exert beneficial of deleterious effects. D. Allan Butterfield, University of Kentucky, USA

Parallel session 12 (1:30pm – 4:00pm, Harbor Ballroom III)

Environmental exposures to nanomaterials: Methods, approaches, detection and modeling
Chairs: Jamie Lead, University of South Carolina, USA, and Teresa Fernandes, Heriot-Watt University, UK

Description: This session covers novel directions in nanoecotoxicology and environmental science. The topics to be covered include environmental processes and exposures as they affect bioavailability and toxicity in natural systems, and addresses novel ('next generation') nanomaterials and their systems including nanocomposites and nanohybrids.

Program:

1:30pm	Contrasting hazard of emerging nanomaterials across a range of species and endpoints – lessons for nanosafety Teresa Fernandes, Heriot-Watt University, UK
1:55pm	Transformations and biological impact of emerging energy storage nanomaterials. Robert Hamers, University of Wisconsin, USA
2:20p0m	Biological and environmental transformations of emerging 2D materials and nanohybrids Robert Hurt, Brown University, USA
2:45pm	Environmental applications of graphene oxide nanoarchitectures. Chad Vecitis, Harvard University, USA
3:10pm	New developments in understanding nanomaterial dose. Jamie Lead, University of South Carolina, USA
3:30pm	Trophic transfer and bioaccumulation of carbon nanotubes in a microbial food chain. Monika Mortimer, University of California, Santa Barbara, USA
3:40pm	Head-to-head comparison of in vitro cytotoxicity and phytotoxicity of antimicrobial mixed-valence Cu nanocomposites with commercially available Cu pesticides. Swadeshmukul Santra, University of Central Florida, USA
3:50pm	Nano-waste: Environmental health and safety implications during thermal degradation/incineration of nano-enabled products at their end-of-life. Dilpreet Singh, Harvard University, USA

Nanoceria: Benefits and risks workshop

9:30am – 4:00pm, Burroughs Room

Chair: Robert A. Yokel, Ph.D.
Co-chairs: Philip Demokritou, Ph.D., Barbara Karn, Ph.D., William Self, Ph.D., David Warheit, Ph.D.

Objective: This workshop will focus on nanocerias, including the results of studies that elucidate beneficial as well as adverse effects, and physicochemical properties that mediate the effects. This is a follow-up session to a workshop on nanoceria that was held November 2, 2013 in Santa Barbara, California, from which several critical reviews were published in Environmental Science: Nano. Those reviews included data gaps/research recommendations to enhance our understanding of nanocerias' myriad effects, some of which are addressed in this session.
The session will begin with an invited presentation of the findings of a two-year NANoREG inhalation study of nanoceria and barium sulfate. Studies by other groups of the distribution, biotransformation, and effects following pulmonary exposure to cerium and nanocerias will follow. Results of studies elucidating the biological response in target organs and cells to nanocerias will then be presented.
The afternoon session will begin with an overview of nanocerias' benefits and applications, followed by recent findings describing beneficial effects targeting specific disease models and bacteria, and a PBPK model to integrate nanocerias' distribution that mediates the effects. The session will conclude with presentations that focus on physicochemical properties related to nanoceria's biological identity, stability, and response.
All registrants of the 8 International Nanotoxicology Congress are welcome to attend this session.

Program:

9:30am Long-term Inhalation Study with nanomaterials: Effects and lung-burden after chronic inhalation study with ceria and barium sulfate.
Karin Wiench

Distribution, biotransformation, and effects following pulmonary exposure to nanocerias

10:15am Pulmonary distribution of nanoceria: comparison of intratracheal bolus and microspray instillation and dry powder insufflation.
Ramon Molina

10:30am Inhalation toxicity of 5-10 nm cerium dioxide nanoparticles.
Rachel Smith

10:45am Fate of cerium ions in the lungs: In vivo formation of cerium phosphate nanoparticles from instilled cerium chloride.
Uschi Graham

11:00am Where does the transformation of precipitated ceria nanoparticles in hydroponic plants take place?
Yuhui Ma

Biological responses to nanocerias

11:05am In vivo genotoxic effects of uncoated and coated CeO_2 NPs administrated to mice by pharyngeal aspiration.
Julia Catalán

11:20am Cerium oxide nanoparticles moderately enhances the cancer features exhibited by cigarette smoke condensate in a human lung epithelial cell line.
Laura Rubio Lorente

11:25am Time-dependent changes in brain induced by intravenously delivered cerium oxide nanoparticles are consistent with potential pathways discerned by proteomics identification of in vivo serum or plasma proteins adsorbed to nanoceria.
D. Allan Butterfield

11:40am Phenotypic differences in aortic and microvascular endothelial cells influences responses to cerium dioxide nanoparticles.
Valerie Minarchick

11:55am Decreased uptake and enhanced mitochondrial protection underlie reduced toxicity of nanoceria in human monocyte-derived macrophages.
Salik Hussain

The beneficial effects of nanocerias

1:30pm The benefits and applications of cerium oxide nanoparticles.
William Self

1:45pm Subcutaneous cerium oxide nanoparticle delivery affords similar protection as intravenous delivery in murine model of multiple sclerosis.
Karin Heckman

1:50pm Cerium oxide nanoparticles preserve muscle function and increase longevity in the SOD1G93A mouse model of amyotrophic lateral sclerosis.
William DeCoteau

2:05pm Measurement of catalase and oxidase activity of custom-synthesized cerium oxide nanoparticles and assessment of their antioxidant effectiveness in a mouse hippocampal brain slice model of ischemia.
Ana Estevez

2:10pm Cerium dioxide nanoparticles modulate bleomycin induced inflammatory and fibrotic events within the rat lung.
Martin Leonard

2:25pm Effect of pH-Varied cerium oxide nanoparticles on the growth of gram-positive and negative bacteria.
Ece Alpaslan

2:40pm Physiologically based pharmacokinetic modeling of cerium dioxide nanoparticles infused intravenously into rats.
Ulrika Carlander

Physicochemical properties related to nanocerias' biological identity, stability and response

2:55pm In vitro toxicity of nanoceria: effect of coating and stability in biofluids.
Jean-Francois Berret

3:10pm Aspect ratio plays a role in the hazard potential of CeO_2 nanoparticles in cells, mouse lung and zebrafish gastrointestinal tract.
Tian Xia

3:25pm Quantitative analysis of the physicochemical properties of cerium oxide nanomaterials and their influence on nano-bio interactions.
Christopher Sims

3:40pm Acquired superoxide-scavenging ability of ceria nanoparticles.
Xiao He

Saturday June 4, 2016
8:00am – 9:00am Plenary III:

Nanoscience approaches to heterogeneity in biological systems

Speaker: Paul S. Weiss. California NanoSystems Institute, University of California, Los Angeles, USA.

Description: The great promise of single-molecule/assembly measurements is to understand how critical variations in structure, conformation, and environment relate to and control function. New approaches to imaging and analysis are keys to elucidating these associations. I will discuss current and upcoming advances and will pose the challenges that lie ahead in creating, developing, and applying new tools for biology and medicine. These advances include fusing spectroscopic imaging modalities and freeing up bandwidth in measurements to record simultaneous data streams and to expand our dynamic range. Recent advances in sparsity and compressive sensing can be applied both to new analysis methods and to directing measurements so as to assemble and to converge structural and functional information. Early examples will be discussed.

Biosketch: Paul S. Weiss leads an interdisciplinary research group that includes chemists, physicists, biologists, materials scientists, mathematicians, electrical and mechanical engineers, and computer scientists. Their work focuses on the ultimate limits of miniaturization, exploring the atomic-scale chemical, physical, optical, mechanical, and electronic properties of surfaces and supramolecular assemblies. He and his students have developed new techniques to expand the applicability and chemical specificity of scanning probe microscopies. They have applied these and other tools to the study of catalysis, self- and directed assembly, and molecular and nanoscale devices. They work to advance nanofabrication down to ever smaller scales and greater chemical specificity in order to operate and to test functional molecular assemblies, and to connect these to the biological and chemical worlds. He has written over 300 publications, holds over 20 patents, and has given over 600 invited, plenary, keynote, and named lectures.
Weiss received his S.B. and S.M. degrees in chemistry from MIT in 1980 and his Ph.D. in chemistry from the University of California at Berkeley in 1986. He was a postdoctoral member of technical staff at Bell Laboratories from 1986-1988 and a Visiting Scientist at IBM Almaden Research Center from 1988-1989. He is a distinguished professor of chemistry & biochemistry and materials science & engineering at UCLA. From 2009-2014, he was the director of the California NanoSystems Institute and Fred Kavli Chair in NanoSystems Sciences. Before coming to UCLA, he was a distinguished professor of chemistry and physics at the Pennsylvania State University, where he began his academic career as an assistant professor in 1989. He is the founding and current editor-in-chief of *ACS Nano*.

Parallel Sessions 13 through 15

Parallel session 13 (9:30am – 12:00pm, Harbor Ballroom I)

Lessons from the real world: What is human nanotoxicology telling us?
Co-chairs: Dhimiter Bello, University of Massachussetts, Lowell, USA and Alison Elder, University of Rochester, USA

Description*:* The main purpose of this session is to compare and contrast experimental nanotoxicology with real-world studies of human health effects following exposures to ENM. The session will start with a summary of major findings from human exposure assessment studies for these scenarios and dose estimates to provide context for the subsequent talks, followed by an overview of biokinetics of nanoparticles in and beyond the lungs. Subsequent presentations will provide a detailed account of findings from real-world human nanotoxicology and molecular epidemiology research for important commercial ENM, and contrast such findings to experimental and mechanistic nanotoxicology database for such materials.

Program:

9:30am	Framing the problem Co-chairs, Dhimiter Bello and Alison Elder
9:45am	All swans are white: what we don't know about biologic responses to inhaled nanoparticles Prof. Joseph Brain, Harvard University, USA
10:20am	Biomolecular effects of TiO_2 exposures in humans: what did nanotoxicology tell us? Prof. Daniela Pelclová, Charles University, Prague, Czech Republic
10:55am	Engineered nanoparticles emitted from laser printers: A case study of environmental health implications from nano-enabled products during consumer use. Dr. Sandra Pirela Harvard University, USA
11:30am	Concepts of dosimetric modeling of rodent inhalation studies with inhaled nanoparticles: The need for allometric scaling of respiratory parameters for extrapolation to humans. Prof. Günter Oberdörster, University of Rochester, USA

Parallel session 14 (9:30am – 12:00pm, Harbor Ballroom II)

Nanomaterials in food and agriculture
Co-chairs: Hongda Chen, USDA, USA and Philip Demokritou, Harvard School of Public Health, USA

Description: Applications of nanotechnology for addressing important societal challenges facing agriculture and food systems have been actively explored worldwide over the past two decades. Nanotechnology is envisioned to provide innovative solutions for sustainable agricultural production, biobased economy, food safety and quality, human nutrition, environmental and ecological systems, natural resources, water quality, and adaptation and mitigation of climate changes. Safety implication of nanotechnology applications and nanomaterials in agricultural products and food have also been studied. This session, for the first time on this platform, will introduce several selected examples of applications and their implications of nanomaterials in food to the community of the Int'l Congress of Nanotoxicology. The main purpose of this session is to raise an awareness of the broad scope of nanotechnology R&D opportunities in agriculture and food. The audience will also learn some important outcomes from these selected studies. It is hoped that the session will attract the participating scientists and researchers of the nanotoxicology community and facilitate productive and collaboration discussions with the broad agricultural nanotechnology R&D community.
This session is designed to showcase some examples of nanotechnology applications in food and agriculture. These include controlling fresh produce safety against food borne pathogens, transformation and transport of lipid nanoemulsions in human GI tract for improving nutrition and health, biodistribution of polymeric vehicles for delivery bioactives in functional foods, food additives, and agricultural crop production. Diverse nanomaterials studied include novel engineered-water-nanostructures (EWN), lipid, polymer, and inorganic nanoparticles. Various advanced and innovative analytical methods and bio-assesses (in-vivo and in-vitro) will also be highlighted.

Program:

9:30am	Introductory remarks, Co-chairs, Hongda Chen and Philip Demokritou
9:55am	Nanotechnology to the rescue: A chemical free, antimicrobial platform using Engineered Water Nanostructures Georgios Pyrgiotakis, Harvard University, USA
9:58am	Lipid nanoparticles in food and gastrointestinal tract: implication in food function and safety Hang Xiao, University of Massachusetts, Amherst, USA
10:21am	Toxicity and *in vivo* distribution of ingested food-relevant inorganic nanoparticles W. James Waldman, Ohio State University, USA
10:44am	Biodistribution and toxicity of orally administered poly (lactic-co-glycolic) acid nanoparticles to F344 rats for 21 days Cristina M. Sabliov, Louisiana State University, USA
11:07am	Characterization of Engineered Nanoparticles and their Behavior and Toxicity in the Gastrointestinal Tract Mengshi Lin, University of Missouri, USA
11:30am	Functional nanotoxicology applied to crop species exposed to engineered nanomaterials Luca Pagano, University of Massachusetts, USA
11:53am	Discussion Hongda Chen and Philip Demokritou

Parallel session 15 (9:30am – 12:00pm, Harbor Ballroom III)

Lessons from Mouse Models of Susceptibility to ENM-Lung Disease
Session Chairs: James Bonner, North Carolina State University, USA and Andrij Holian, University of Montana, USA

Description: Immunotoxicity caused by exposure to engineered nanomaterials (ENMs) is a growing concern for human health. This symposium will cover progress made in understanding mechanisms of ENM-induced immunotoxicity following pulmonary exposure as well as susceptibility issues. Topics covered will include mechanisms of ENM-induced innate immune responses in the lung including macrophage inflammasome activation and genetic strain variation of ENM-induced lung inflammatory response emphasizing the importance of genetic susceptibility. The physico-chemical properties of ENMs that predict lung inflammation in rodents and humans will be discussed. Also, susceptibility to ENM immunotoxicity caused by pre-existing allergen-induced lung inflammation will be discussed. It is anticipated that audience will gain a broad perspective of environmental and genetic factors that determine ENM immunotoxicity in the lung.

Program:

9:30am	Genetic determinants of nanoparticle lung inflammation Terrance Kavanagh, University of Washington, USA
10:00am	Multi-walled carbon nanotube physicochemical properties predict pulmonary inflammation and genotoxicity Sarah Søs Poulsen, National Research Centre for the Working Environment, Denmark
10:30am	Phagolysosomal membrane permeability and inflammasome activation as host susceptibility factors in ENM-induced lung inflammation Andrij Holian, University of Montana, USA
11:00am	Differential susceptibility in humans and rodent models of nanomaterial toxicity Salik Hussain, National Institute of Environmental Health Sciences, North Carolina, USA
11:30am	Susceptibility to ENMs in mouse models of allergen-induced lung disease James Bonner, North Carolina State University, USA

Poster presentations

Poster presenters are asked to set up their posters in the morning of Thursday June 2nd. Posters will remain available for viewing till Friday 6:00pm. Poster presenters from group I should be available to present their posters on Thursday, June 2nd from 4:30-5:30pm. Group 2 poster presenters will present their posters on Friday, June 3rd from 4:30-6:00pm. Any posters left on the boards will be discarded after 6:00pm on Friday.

Please also note that a number of poster awards will be given including a $500 cash prize for best poster sponsored by Nano-IMPACT journal

Group I posters 1 through 100

1. Vascular Response Gradient Elicited by Acute Nano-Titanium Dioxide Exposure

Alaeddin Abukabda, Timothy Nurkiewicz, Phoebe Stapleton. West Virginia University, USA.

Engineered nanomaterial (ENM) exposures have been associated with adverse cardiovascular outcomes. However, the disparity of these results warrants a segmental assessment of the systemic vasculature to determine the most sensitive levels where these effects manifest. Wire-myography was used to evaluate active tension generation in the thoracic aorta, femoral artery and mesenteric arterioles from Sprague-Dawley rats. Denudation via physical abrasion was performed in order to assess endothelial influence post-exposure. Nano-TiO_2 with a primary particle size of 21 nm was utilized for this study. Two exposure routes were tested:coincubation (30 minutes; 20, 100 and 200 µg per 250 µl of medium) and pulmonary via intratracheal instillation [(24 hours prior; 24, 120, or 240 µg suspended in 300 µl of vehicle)]. Rings were precontracted with phenylephrine (PE; $1x10^{-3}$M) and challenged with acetylcholine (Ach; $1x10^{-9}$-$1x10^{-4}$M), and sodium nitroprusside (SNP; $1x10^{-9}$-$1x10^{-4}$ M). Instillation of 240 µg nano-TiO_2 decreased aortic and femoral artery ACh response by 46±4% and 47±6%, respectively. Mesenteric arteriolar cholinergic reactivity was reduced by 96±3%. Coincubation with 200 µg of nano-TiO_2 reduced aortic responsiveness to ACh by 37±9%, while augmenting α-adrenergic response by 43±7%. Femoral artery and mesenteric arteriole cholinergic reactivity was diminished by 61±7% and 94±5%, respectively. Endothelial denudation resulted in similar responses between denuded control and exposed rings. These results indicate that endothelial dysfunction increases in severity moving toward the resistance vasculature.

Keywords: Metal/metal oxide nanomaterials, Occupational safety, endothelium, vascular reactivity

2. The Chamber System for Nanoparticle Release from Nanocomposite

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With the rapid development of nanotechnology as one of the most important technologies in the 21st century, interest in the safety of consumer products containing nanomaterials is rapidly increasing. Evaluating the nanomaterial release from products containing nanomaterials is a crucial step in assessing the safety of the products containing nanomaterials, and has resulted in several international efforts to develop consistent and reliable technologies for standardizing the evaluation of nanomaterial release. In this study, the release of nanomaterials from products containing nanomaterials is evaluated using a chamber system that includes a condensation particle counter, optical particle counter, and sampling ports to collect filter samples for electron microscopy analysis. The proposed chamber system is tested using abrasor and disc-type nanocomposite material specimens to determine whether the nanomaterial release is repeatable and consistent within an acceptable range. The test results indicate that the total number of particles in each test is within 20% from the average after several trials. The release trends are very similar and showed very good repeatability. Therefore, the proposed chamber system can be effectively used for nanomaterial release testing of products containing nanomaterials.

Keywords: Methods, Nanomaterial release, Nanoparticles, Nanomaterials, Release, Nanocomposites, Simulation, Chamber

3. Signaling Mechanisms of Silver Nanoparticle-Mediated Activation of Mast Cells

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Engineered nanomaterial-mediated toxicity often involves triggering immune responses that includes mast cell (MC) activation. MC can regulate both innate and adaptive immune responses and are key contributors to allergic responses and inflammation. Silver nanoparticles (AgNPs) are one of the most prevalent nanomaterials used in consumer products (e.g. electronics, detergents, coating of medical devices, etc.) due to their antimicrobial/antifungal properties. We utilize bone marrow-derived MC isolated from C57Bl/6 mice. We have previously shown that AgNPs induce MC degranulation, which is dependent on nanoparticle physicochemical properties. Furthermore, we identified a role for scavenger receptor B1 (SR-B1) in AgNP-mediated MC degranulation. However, it is completely unknown how SR-B1 mediates MC degranulation and the intracellular signaling pathways involved. We hypothesized that SR-B1 interaction with AgNPs directs MC degranulation through activation of phosphoinositide 3-kinase (PI3K) and phospholipase Cγ (PLCγ) and subsequent influx of extracellular calcium through the ORAI calcium channels. We found that AgNP-mediated MC degranulation is dependent on an influx of extracellular calcium, which appears to be at least partially mediated by the ORAI1 calcium channels. Moreover, we found that both PI3K and PLCγ are involved in degranulation of MCs in response to AgNPs. Taken together, our results provide new insights into AgNP-induced MC degranulation, which is beneficial for designing novel nanomaterials that are devoid of immune system activation.

Keywords: Adverse outcomes pathway analysis, In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

4. Aberrant Phenotype on Chronic Exposure of Zinc Oxide Nanoparticles: A Study on Drosophila Melanogaster

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Nanotechnology is an upcoming endeavor with potential of distinctive properties. Zinc oxide (ZnO) nanoparticles (NPs), a commonly used nano material, the widespread use has brought human in close interface and it is essential to understand toxicity caused by these NPs . Drosophila melanogaster is one of the most effective model organism to study chronic effect of NP exposure because of its simple anatomic features and physiology. We have assessed the effect of chronic exposure of ZnO NPs on model organism Drosophila melanogaster (fruit fly). Set of ten virgin female and male flies were exposed to ZnO NPs via ingestion at various concentration (0.1 mM to 10 mM) of NPs dispersed in fly diet. To ensure uniform distribution of the particles and size in accordance to the dose, dynamic light scattering and transmission electron microscopy was performed. Potential toxic effects were studied via longetivity, climbing ability, immunologic response, oxidative stress and TUNEL assay. Ensuing exposure the F0 (parent), F1, F2 and F3 generation flies were screened for aberrant phenotype. Distinctive phenotypic changes were observed in flies, with deformed segmented thorax and single wing or deformed wing which was transmitted to the offspring in the subsequent generations. Deformed wings were observed in most of the flies, an indicative of mild phenotypic deformation whereas severe phenotypic changes viz. pigmented, distorted and segmented thorax was observed in restricted number of flies. Unique abnormal phenotype is evident of chronic toxicity induced by ZnO NPs, although alarming but emphasize to understand NPs toxicity.

Keywords: Developmental nanotoxicology, Genotoxicity, In vivo toxicology, Metal/metal oxide nanomaterials, Systems biology/toxicology

5. Bioreactors Experiments Coupled with Membrane Permeability and Langmuir Balance Assays to Assess the Inhibition of Nanoparticles on E. coli

Nelson Anaya, Fatemeh Faghihzadeh, Nasim Ganji, Geoff Bothun, Vinka Oyanedel-Craver. University of Rhode Island, USA.

Chemostat and batch reactors were used to study the response of Escherichia coli (E. coli) exposed to casein-coated silver nanoparticles (AgNPs) from 1 to 50 mg/L during 12.5 hours after steady conditions. Batch tests were run using a microplate at similar conditions to the chemostat tests. Bacteria membrane extracts, membrane permeability and Langmuir film balance assays were used to determine integrity and changes in lipid composition in response to AgNPs exposure. Results showed batch conditions were not appropriated for the tests due to the production of exopolymeric substances (EPS) during the growth phase. After 5hours of contact between AgNPs and the used growth media containing EPS, AgNPs increased in size from 86 to 282 nm, reducing the stability, thus limiting cell-nanoparticle interactions. Membrane extracts assays showed that 1 mg/L AgNPs had a higher change in area (ΔA) (-4.4 cm^2) on bacteria compared to 15mg/L (-4.0 cm^2). This ΔA suggested a membrane disruption caused by AgNPs and bacteria responded by shifting their lipid composition to more unsaturated lipids to counteract membrane rigidification. In chemostats, the constant inflow of fresh media and aeration resulted in less AgNPs aggregation, thus increased the AgNPs-bacteria interactions, in comparison to batch conditions. AgNPs membrane extract exposed to 1, 15, and 50 mg/L of AgNPs showed higher ΔA by -0.5, 2.7 and 3.6 cm^2, respectively, indicating that the bacterial membranes were disrupted and bacteria responded by synthesizing lipids that stabilize or strengthen their membranes. This study showed that the chemostat is more appropriate for testing of nanotoxicological effects when testing bacteria at growing conditions. Funding provided by NSF.

Keywords: Developmental nanotoxicology, Environmental nanotoxicology, In vitro toxicology, Toxicological mechanisms

6. Effect of Titanium Dioxide Nanoparticles on the Mobility of Lipids in Model Membranes and Cells

Donald Anderson, Harmen B. Steele, J.B. Alexander Ross, Andrij Holian. University of Montana, USA.

The proliferation of nanotechnology is resulting in the increased production and use of engineered nanomaterials (ENM). This rapid expansion of nanotechnology poses an increased risk of human exposure to ENM. One type of ENM whose use has increased greatly are titanium dioxide nanoparticles (TiO_2-NP). While spherical TiO_2-NP (TiO_2-NS) have been shown to be non-toxic, TiO_2-NS are taken-up by phagocytic cells where they can accumulate. This provides an opportunity for these particles to interact with cellular lipid membranes. To investigate the relationship between TiO_2-NP and lipid membranes we used a supported lipid bi-layer model and live cells. Our model membrane system consisted of 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) (99.5% mol/vol) and 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) (0.5% mol/vol) lipid bilayer supported on cleaned and bovine serum albumin coated coverslips. Live cells were bone marrow derived macrophages (BMdM) obtained from mice. The fluorescent membrane probe Di-4-ANEPPDHQ was used to determine the fluidity of BMdM lipid membranes. Lipid fluidity was measured using fluorescence recovery after photobleaching (FRAP) on an Olympus FV-1000 inverted microscope. Analysis of collected FRAP data was performed using Easy FRAP software. A significant decrease in FRAP recovery time ($t_{1/2}$) in the lipid by-layer model treated with TiO_2 was observed within 2 hr. Furthermore, a trend toward decreased $t_{1/2}$ was observed within 2 hr using BMdM. Taken together, these results support the notion that NP have defined impacts on membrane properties that may account for initial mechanisms of toxicity. Funding provided by NIH R01ES023209 and P30GM103338.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms,

7. Autophagy Related Gene Regulates the Uptake of Silica Nanoparticles

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We have previously found that silica nanoparticles might induce autophagy that plays an important role in defense against the foreign substances such as bacteria, virus, or nanoparticles. On the other hand, it is suggested that autophagy related gene (ATG) proteins regulate not only host defense but also membrane dynamics such as endocytosis. Endocytosis is reported as the uptake pathway of nanoparticles. Thus, endocytosis controlled by autophagy and ATG proteins may affect the uptake of nanoparticles. However, there are few reports about the relationship between dynamics of silica nanoparticles and autophagy or ATG proteins. Therefore, we examined the contribution of autophagy and ATG proteins to the uptake of silica nanoparticles. We assessed the uptake of silica nanoparticles in autophagy-defected cells expressing the mutant of ATG protein and ATG proteins knockdown cell. As a result, the uptake of silica nanoparticles was significantly inhibited by defection of autophagy or knockdown of ATG proteins. Interestingly, the knockdown of one ATG protein inhibited the uptake of silica nanoparticles regardless of activation of autophagy. These results suggest that ATG proteins could regulate the uptake of silica nanoparticles without activation of autophagy. We are now studying the mechanism how ATG proteins control endocytosis to resolve the relationship between nanoparticles' dynamics and autophagy.

Keywords: Biodistribution, Composite nanomaterials, In vitro toxicology, Toxicological mechanisms

8. Modulation of the Physiological and Biochemical Effects of Copper Nanoparticles in Kidney Beans (Phaseolus vulagaris) Treated with Kinetin

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Engineered nanoparticles (NPs) have become ubiquitous in modern technology. The full scale of their environmental impact is not well understood. Moreover, complete life cycle studies on the toxicity of NPs to crop plants is lacking. Copper (Cu) NPs become an emerging solution towards fungal and bacterial remediation due to their antimicrobial properties. Additionally, plant growth hormones are also being sought as a bio-based alternative to improve crop quality and yield. The high protein content of kidney beans (Phaseolus vulagaris) renders it an important food crop that is widely produced and consumed worldwide. In this study, kidney bean plants were grown in potting soil treated with copper nanoparticles (nCu), bulk copper (bCu) and copper chloride ($CuCl_2$) at concentrations of 50 and 100 mg/kg-1 for 40 and 100 days, respectively. At 15 days of growth, 10 and 100 µm of kinetin (KN) was applied to plants. Plant tissue samples were taken during the juvenile stage, and seeds were collected at the time of maturity. Physiological and biochemical parameters were investigated. Cu uptake, translocation and micronutrients were determined via inductively couple plasma-optical emission spectroscopy (ICP-OES). Enzyme activities and chlorophyll content were evaluated as plant stress indicators. Seed macronutrients were also quantified. Results indicated that Cu and KN treatments did not significantly affect agronomic parameters or crop yield. For future work, KN content in soil and plant tissues will be studied. Funding provided by UC-CEIN.

Keywords: Environmental nanotoxicology, Metal/metal oxide nanomaterials, Risk assessment, Copper nanoparticles, Kinetin, Kidney bean, ICP-OES, Antioxidant enzymes

9. Toxicological Differences between Pristine and Agin PVP and Silica Coated Ag Nanoparticles in Aquatic Ecosystems

Ester Artells[1], Clement Levard[2], Julien Issartel[1], Melanie Auffan[2], Alain Thiery[1], Jerome Rose[2]. [1]IMBE-AMU, [2]CEREGE-CNRS, France.

In the past 20 years, recent advances in nanotechnology have resulted in the generation of various nanostructured materials, which have unique physical and chemical characteristics. The engineered nanomaterials (ENMs) production is increasing rapidly and have been introduced in our daily lives. Silver nanoparticles (AgNPs) are the most extensively used nanoparticles in consumer products principally because their antibacterial properties. During manufacture, use or end of life of NPs, a significant amount or NPs can be released into the environment, however, the environmental release of NPs, the accumulation of these new polluants in hydrographic basins and their effects on aquatic organisms are currently unclear. Understanding the toxic effects of these emerging xenobiotics is therefore crucial in order to anticipate the consequences of the potential transformations of nanomaterials in ecosystems and their potential impact on health. In a "safer by design" perspective, this project propose a multiapproach analysis regarding the potential transformations of AgNPs coated with either silica or PVP in environmental conditions. This project combine physico-chemical analyses (DLS, ICP, TEM, X-Ray microtomography...), as well as biological and toxicological analyses (survival and behavioral studies, histology, gene expression, protein interactions...). Preliminary results shows both information regarding NPs evolution in time and changes in their putative toxicity between pristine and aging NPs. This work is part of the SERENADE (Safe(r) Ecodesing Research and Education applied to Nanomaterial Development) project whose goal is to create a dynamic network of academic research laboratories and industry to design tomorrow's nanomaterials that are safer for both humans and environment.

Keywords: Environmental nanotoxicology, Exposure characterization, Metal/metal oxide nanomaterials, Physicochemical characterization.

10. Ecotoxicology of Sediment-associated Carbon Nanotubes

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Carbon nanotubes (CNTs), single-walled (SWCNTs) and multi-walled (MWCNTs), are high aspect ratio nanostructures with a combination of properties making them useful in an increasing number of products and applications. Few in vivo studies have examined the behaviour of CNTs in marine systems and their bioavailability and toxicity to marine organisms. We have previously shown that SWCNTs show relatively low toxicity to marine mussels; the main concern was that SWCNTs influence the toxicity of other contaminants at otherwise benign concentrations. CNTs in the aquatic environment are expected to rapidly precipitate and become incorporated into sediments. The aim of the present study was to assess the behaviour of CNTs in sediments, their bioavailability to benthic species and their effect on the bioavailability and toxicity of other sediment-associated contaminants. Preliminary experiments showed that CNTs (500 μgL^{-1}) could be recovered and confirmed using Raman spectroscopy (RS), albeit with very weak spectra; we are currently improving the technique. Ecotoxicological effects of sediment-associated SWCNTs and MWCNTs (dispersed in 0.02% SRNOM) were investigated by injecting them into seawater tanks (pH7.9-8; 20°C) containing light-coloured play pen sand washed (three times with distilled water and leave it to dry) play pen sand. CNTs were left to settle on to the sediment surface, after which cockles (Creastoderma edule) were introduced and left to bury and filter-feed the nephloid layer for 72hrs. Uptake of CNTs was confirmed using RS and TEM imaging. A suite of biomarkers of exposure were studied, including oxidative stress (SOD, CAT, TBARS, GSH, GPx) and associated genotoxicity. CNTs settled down very quickly after one hour and no changes in their behaviour were observed after 24 hours. Early genotoxicity data suggest that sediment-associated CNTs caused an increase in DNA strand breaks in C. edule at concentrations >100 μgL^{-1}. Work is currently ongoing to extend the exposure concentration range down to more environmentally relevant levels and to test the causative oxidative stress hypothesis.

Keywords: Environmental nanotoxicology, SWCNTs, MWCNTs, clams, cockles, Cersstoderma edule, marine sediments, ecotoxicology, sediment

11. Epigenetic Changes of Ag_2S Quantum Dot Coated with Meso-2,3-dimercaptosuccinic Acid of Related to Apoptotic Pathway

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The increasing interest in nanotechnology has contributed to the development of new products and their applications in various sectors. Since the toxicological issues of nanoparticles are very important, many investigations on nanoparticles have been performed in the area of nanotoxicology. In limited studies, some nanoparticles have been shown to be able to change the expression of some apoptotic genes. Silver containing quantum dot (QD) nanoparticles due to having very small size, great surface area have impressive characteristics and useful applications such as electronics, biosensors. It has been suggested to be useful for imaging of cancer cells due to special optical properties and photoelectrochemical activity. However there are limited studies on the effects of silver sulfide quantum dots coated with meso-2,3-dimercaptosuccinic acid (Ag_2S ODs-DMSA) on apoptotic pathways. In this study, it was aimed to determine the differences in the expression of some apoptosis-related genes in Chinese lung fibroblast (V79) cells treated in a wide concentration range of (10, 40,125, 500 µg/ml) of Ag_2S ODs-DMSA by real-time polymerase chain reaction (PCR) assay. It was found that Ag_2S ODs-DMSA changed the expression of genes (caspase 9, bax, bcl2) related to apoptotic pathways. The further detailed studies using more genes related to apoptosis are needed. The investigation of possible apoptotic mechanisms of QDs will contribute to the safety uses of QDs. Funding provided by TUBITAK (contract grant number: 114S861).

Keywords: In vitro toxicology, Toxicological mechanisms, meso-2,3-dimercaptosuccinic acid (Ag_2S ODs-DMSA), Chinese lung fibroblast (V79) cells, nanotoxicology, apoptosis

12. Carboxylated Multi-walled Carbon Nanotubes Enhance Ovalbumin directed Macrophage activation and CD4+ T cell proliferation

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Carbon nanotubes have been of great interest for drug and vaccine development due to their unique physicochemical properties. The high surface area to volume ratio of carbon nanotubes promotes binding of proteins to the surface forming a protein corona. Based on previous work that demonstrated increased protein binding, we propose that carboxylated multi-walled carbon nanotubes (MWCNTs) can function as an improved carrier to deliver antigen such as ovalbumin (OVA). To test this hypothesis, we coated carboxylated MWCNTs with ovalbumin and measured uptake and activation of antigen-presenting cells (macrophages) and their ability to stimulate CD4+ T cell proliferation. We employed two types of carboxylated MWCNTs with differential dimensions, including MWCNT-1 (10-20 nm, 0.5-2 μm) and MWCNT-2 (10-20 nm, 10-30 μm). The two MWCNTs bound different amounts of OVA with MWCNT-1 binding 582 ± 41μg/ml and MWCNT-2 binding 1066 ± 182μg/ml . MWCNTs showed no observable toxicity to bone marrow-derived macrophages (BMDM) up to 5 days. Surprisingly, we found that MWCNT-OVA complex significantly increased the expression of major histocompatibility complex class II (MHCII) on macrophages, and production of pro-inflammatory cytokines (TNFα and IL-6), while MWCNTs without OVA protein corona did not. The co-culture of MWCNT-OVA complex treated macrophages and ovalbumin-specific CD4+ T cells isolated from OT-II mice demonstrated robust proliferation of T cells. Our work provides a strong evidence for MWCNT to act as an antigen delivery vehicle in the development of next-generation vaccines. Funding provided by U19 ES019525, R03 ES023036, and the NIEHS Centers for Nanotechnology Health Implications Consortium.

Keywords: Carbon-based nanomaterials

13. Sustained Inflammation and Genotoxicity following Pulmonary Exposure to Graphene and Graphene Oxide

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In the present study, pulmonary inflammation and genotoxicity of thoroughly characterized commercially-available graphene oxide (GO) and reduced graphene oxide (rGO) were evaluated in vivo. GO and rGO consisted mainly of 2-3 stacked graphene sheets (>1 µm). The molar content of oxygen in relation to carbon (C/O ratio) was 1.4 and 12.8 for GO and rGO, respectively. Both had low levels of inorganic impurities and endotoxin.GO or rGO was instilled into lungs of female C57BL/6 mice by single intratracheal instillation at three doses (18, 54 or 162 µg/animal). Pulmonary inflammation and genotoxicity in broncho-alveolar lavage (BAL) fluid, lung and liver were assessed after 1, 3, 28 and 90 days. Inflammation was assessed by differential cell counting by neutrophil influx in BAL fluid. The level of DNA strand breaks assessed with the comet assay was used as marker for genotoxicity.GO and rGO induced prolonged inflammation. GO induced strong inflammatory response at day 1 and 3. rGO induced less inflammation compared to GO. However, increased neutrophil influx in BAL fluid was still observed after 90 days for both GO and rGO. In addition, increased level of DNA strand breaks in BAL cells was observed across days and doses. No increased level of DNA strand breaks was observed in lung and liver tissue. These results suggest that GO is more inflammogenic than rGO, whereas both induce long-lasting inflammation and genotoxicity in BAL cells in vivo. Funding provided by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n°FP7-604000.

Keywords: Carbon-based nanomaterials, Genotoxicity, In vivo toxicology, Physicochemical characterization

14. A "Safety-by-Design" Approach for risk management of Engineered Nanomaterials (ENM): Evidences from the EU Project SaNoWork

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In spite of the concerns raised by the nanotechnology innovation, there is an increasing consensus that material scientists, business leaders and health and safety professionals should converge efforts to develop and implement safer manufacturing processes. Since elimination/substitution is unlikely to be a safety option, effective risk mitigation strategies can rely on the "safety-by-design" (SbD) approach to proactively prevent hazard and exposure potential, while at the same time preserving the innovative properties of ENM. The EU-FP7 Project SaNoWork (NMP4-SL-2012-280716) applied this principle to industrial processing lines manufacturing nanoparticles of ZrO_2, TiO_2, Ag, MWCNTs and TiO_2 nanofibers. Following an ENM-specific SbD approach, the surface or structure of the ENM was changed, and the effect of remediation on hazard and/or exposure potential evaluated, along with changes in performance, in pristine and remediated forms. Simple cost effective measures and organizational changes could be deployed such that the exposure and hazard potentials were significantly reduced, e.g. using well-established freeze and spray drying procedures, which do not significantly lower the functionality of the materials involved. Since a certain level of occupational risk is present in several of the processing lines, to demonstrate its attenuation would have a great value for regulators or insurers tasked with a risk assessment of similar manufacturing environments. Such an a priori mitigation of potential hazard should reduce the need to manage possible health consequences coming from exposure, especially if prolonged. However, the effectiveness of this approach should rely on a thorough, case-by-case assessment of both safety issues and ENM performance.

Keywords: Occupational safety, Risk Management

15. Preventing Corona Effects: Multi-phosphonic Acid Poly(ethylene glycol) Copolymers for Stable Stealth Iron Oxide Nanoparticles

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When dispersed in biological fluids, engineered nanoparticles are selectively coated with proteins, resulting in the formation of a protein corona. Recent reports describe this phenomenon as ubiquitous and independent of the nature of the particle. For nanomedicine applications gowever, there is a need to design advanced and cost-effective coatings that are resistant to protein adsorption and that increase the biodistribution in vivo. In this study, phosphonic acid poly(ethylene glycol) copolymers were synthesized and used to coat iron oxide particles [1,2]. The copolymer composition was optimized to provide simple and scalable protocols as well as long-term stability in culture media. It is shown that polymers with multiple phosphonic acid functionalities and PEG chains outperform other types of coating, including ligands, polyelectrolytes and carboxylic acid functionalized PEG. PEGylated particles exhibit moreover exceptional low cellular uptake, of the order of 100 femtograms of iron per cell [2]. The present approach demonstrates that the surface chemistry of engineered particles is a key parameter in the interactions with cells. It also opens up new avenues for the efficient functionalization of inorganic surfaces.

[1] L. Qi et al., ACS Nano 2, 879 (2008); [2] V. Torrisi et al., Biomacromolecules 15, 3171 (2014).

Keywords: Biocorona, In vitro toxicology, Methods, Physicochemical characterization

16. Acetyl-L-carnitine Rescues Pulmonary Mitochondrial Dysfunction in the Metabolomics study of Aluminum Oxide Nanoparticles

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Background: Due to the wide application of engineered aluminum oxide nanoparticles (Al_2O_3 NPs) , exposure of human to nano-scale Al_2O_3 NPs is becoming inevitable. Methods: RNA microarray coupled with metabolomics analysis were used to uncover mechanisms underlying cellular responses to Al_2O_3 NPs and imply the potential rescue. Subsequently, modulation of gene expression in human bronchial epithelial (HBE) cells and mice lung tissues were verified by qRT-PCR assays. Mitochondrial functions and rescue of Acetyl-L-carnitine (ALCAR) were assessed through alterations of caspase-3, 8 and 9 activity, mitochondrial membrane potential ROS and MDA in Al_2O_3 NPs treated HBE cells and mice lung tissues. Results: We found that Al_2O_3 NPs significantly triggered down-regulation of mitochondria-related genes located in complex I, IV and V in HBE cells. Subsequent cell- and animal-based assays confirmed that Al_2O_3 NPs caused mitochondria-dependent apoptosis and oxidative stress either in vitro or in vivo, which were consistent with the trends of gene regulation. To rescue the Al_2O_3 NPs induced mitochondria dysfunction, disruption of small molecular metabolites of HBE were profiled using metabolomics analysis. Supplementation of an antioxidant, ALCAR, completely or partially restored the Al_2O_3 NPs modulated gene expression levels in mitochondrial complex I, IV and V. It further reduced apoptosis and oxidative damages in both Al_2O_3 NPs treated HBE cells and animal lung tissues. Conclusion: Thus, our results demonstrate the potential mechanism of respiratory system damages induced by Al_2O_3 NPs.

Keywords: Environmental nanotoxicology, Exposure assessment, Risk assessment, aluminum oxide nanoparticles, mitochondria, acetyl-L-carnitine, nanotoxicology, metabolomics

17. Toxicities Associated with the Occupational Life Cycle of MWCNT

Lindsey Bishop[1], Lorenzo Cena[1], Marlene Orandle[1], Vamsi Kodali[1], Matthew Dahm[1], Mary K. Schubauer-Berigan[1], Tina Sager[1], James Scabilloni[1], Diane Schwegler-Berry[1], Tracy Eye[1], Lori Battelli[1], Jonathan Kang[2], Gary Casuccio[3], Kristin Bunker[3], Aleksandr Stefaniak[1], Patti C. Zeidler-Erdely[1], Linda M. Sargent[1], Robert R. Mercer[1], Aaron Erdely[1]. [1]NIOSH; [2]West Virginia University; [3]RJ Lee Group, USA.

Companies synthesize or purchase as-produced multi-walled carbon nanotubes (AP-MW), apply a commercial or proprietary polymer coating (PC-MW), then embed the PC-MW into a composite matrix. Since workers are potentially exposed at each production stage, we evaluated the toxicity profile of these materials. Initially, male C57BL/6J mice aspirated 4 or 40 µg AP-MW or PC-MW using preparations to emulate personal breathing zone collections. Bronchoalveolar lavage and tissues were collected at 4 h, 1, 7, 28 and 84 d post-exposure. While the AP-MW induced dose- and time-dependent measures of pulmonary cytotoxicity and inflammation, applied polymer coatings did not enhance those effects. Histopathologic changes included small granulomas at terminal bronchioles at 84 d but no significant alveolar fibrosis at the 40 µg dose. The material was significantly aggregated and unlike larger diameter multi-walled carbon nanotubes (MW), no translocation to systemic organs was detected. Lastly, composites with 0.15% and 3% PC-MW by weight were subjected to an industrial sanding process. Assessment of generated aerosols show primarily micrometer-sized particles with some MW protrusions. No evidence of free MW was observed. Interestingly, the characteristics of the base composite material itself and the addition of PC-MW affect the particle number, and subsequently, the respirable fraction of the generated aerosol. These findings provide insight on the toxicity at different stages of the MW production and suggest that while the number of people potentially exposed increases along the product value chain during this kind of production process, the potential for MW exposure greatly decreases.

Keywords: Carbon-based nanomaterials, Life cycle analysis, Nanomaterial release, Occupational safety

18. Towards the Multi-element Detection of Nanomaterials

Steffi Böhme, Olga Borovinskaya, Claudia Cascio, Anna Undas, Hans Marvin, Stefan Weigel. RIKILT, Netherlands.

The production and application of engineered nanomaterials (ENM) has grown within the last years. Therefore, the usage of cosmetics, food or other products with ENM leads to an inevitable release of ENM and the accumulation, aggregation, or dissolution of these materials in the environment. Available analytical techniques are often not specifically developed to analyse ENM. For the detection of ENM in complex media, such as food or cosmetics established techniques have to be developed further or new techniques have to be implemented. The presented study focussed the described lack of techniques and analysed industrial applied ENM and ENM in food products in terms of the particle size distribution, particle mass and number concentration by a single particle ICP-MS approach (spICP-MS). The results were verified by dynamic light scattering (DLS) and electron microscopy (SEM+EDX) measurements. Furthermore, a new multi-element spICP-MS method which uses a time-of-flight ICP-MS (ICP-TOF-MS) was applied. With the ICP-TOF-MS we were able to analyse all respective elements in the ENM simultaneously at a high time resolution. Therefore, the acquisition of additional information on the composition of multi-element particles or particles which might be covered with other matrix components (e.g. in environmental samples) became possible. Funding provided by the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme (EU-projects Innanopart and NanoDefine).

Keywords: Emerging nanomaterials, Exposure characterization, Metal/metal oxide nanomaterials, Methods, Physicochemical characterization, Nanoanalytics

19. Pushing Preclinical Research in Nanomedicine: How to Control Flow Cytometry as a Viable Tool for Bio-assessment of Nanoparticles in Early Development

Nils Bohmer[1], Cordula Hirsch[1], Matthias Rösslein[1], Elijah J. Petersen[2], John T. Elliott[2], Harald Krug[1], Peter Wick[1]. [1]Empa-Swiss Federal Laboratories for Material Testing and Research, Switzerland; [2]National Institute of Standard and Technology, USA.

Nanotechnology is one of the key-technologies of the 21st century and has the potential to facilitate new diagnosis and therapy options in nanomedicine. Even though the number of publications in this area has been exponentially increasing for some years now, only a few products have found their way into the clinic. One reason is the lack of testing strategies accepted by regulatory bodies for proper risk assessment. Due to their unique physico-chemical properties nanoparticles often interact unpredictably with classical toxicological assays and therefore influence the robustness of the tests, yielding false-positive or –negative results thus resulting in inconsistent data in the literature. The aim of this study is to investigate the performance of flow cytometry and to identify critical parameters when nanomaterials with different properties are examined. Based on the AnnexinV-PI-assay, a common method for quantitative detection of apoptotic and necrotic cells, possible interferences of nanoparticles are characterized in an adherent (A549) and a suspension (THP-1) model cell line. Furthermore an inter-laboratory test is executed to standardize instrument performance across different manufacturers and laboratories. The final goal is to develop a protocol, including an innovative experimental design, which enables researchers to avoid undesirable influences of nanomaterials on flow cytometry. Hence understanding the behaviour of nanoparticles in in vitro assays will support a reliable risk assessment of nanomaterials and will finally speed up preclinical research in nanomedicine. Funding provided by NanoScreen Materials Challenge co-funded by the Competence Centre for Materials Science and Technology (CCMX), German Federal Ministry for Education and Research (BMBF; 03X0131A).

Keywords: Alternative testing methods/strategies, In vitro toxicology, Methods, Risk assessment

20. Protein Corona and Proteomic Response of Silver Nanoparticles in Intestinal Caco-2 cells

Linda Böhmert[1], Sabine Juling[1], Alicia Niedzwiecka[1], Dajana Lichtenstein[1], Holger Sieg[1], Michael Schümann[2], Sören Selve[3], Albert Braeuning[1], Eberhard Krause[2], Alfonso Lampen[1]. [1]Federal Institute for Risk Assessment; [2]Leibnitz-Institute for Molecular Pharmacology; [3]Technische Universität Berlin, Germany.

Nanotechnology is used in many products. However, potential effects of silver nanoparticle uptake in humans are not fully understood. In a biological environment, for example after uptake into a cell, nanoparticles interact with biopolymers like proteins to form complex surfaces, a process which has an impact on the interaction between nanoparticles and cellular structures. Thus the identity of the proteins that bind to the silver nanoparticles, also termed protein corona, has an impact on the cellular consequences of nanoparticle exposure. We used 1D-SDS-PAGE and LC-ESI-MS/MS to analyze the proteomic response of an in vitro model of enterocytes (Caco-2 cells) exposed to silver nanoparticles for 24 h, as well as the protein corona of these silver nanoparticles after incubation in Caco-2 cell lysate. Ultracentrifugation was used to separate nanoparticles from unbound proteins. The identified 389 and 718 proteins interacting with silver nanoparticles and silver ions in Caco-2 cells respectively were classified according to their biological functions. The functions of the identified proteins correlated with observed proteomic alterations in intestinal cells in response to treatment with silver nanoparticles like oxidative stress and morphological or metabolic changes. The results of this study show that the proteins of the corona of silver nanoparticles and proteins that interact with silver ions are involved in a variety of cellular processes that are affected by silver species.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials

21. Distribution of Silver Nanoparticles and Silver Ions in the Body: All Silver Species are Relevant

Linda Böhmert[1], Sabine Juling[1], Gerald Bachler[2], Dajana Lichtenstein[1], Alicia Niedzwiecka[1], Holger Sieg[1], Sören Selve[3], Albert Braeuning[1], Alfonso Lampen[1]. [1]Federal Institute for Risk Assessment; [3]Technische Universität Berlin, Germany; [2]ETH Zürich, Switzerland.

Silver is an ubiquitously occurring element with no known physiological function in the human body. Due to the bacterized properties of silver ions, silver is used in a wide range of products for which such an effect is desired. Concerning their distribution and toxicity, published literature points towards silver ions released from the particles as mainly relevant species. However, it remains challenging to distinguish between the effect of silver nanoparticles and silver ions, due to the equilibrium between particles, ions and chemical entities produced by reactions of silver with surrounding components. We used a combination of a short-term in vivo study in rats and a literature-based toxicokinetic model to determine tissue distribution and to estimate mixture ratios of the different silver species (nanoparticles, ions and de novo-formed particles) after intravenous injection of silver nanoparticles and silver ions. We present experimental data on organ distribution which are accurately reflected by predictions of PBPK model. PBPK modeling suggests systemic distribution of a major proportion of injected ionic silver as de novo-formed secondary nanoparticles and electron microscopy confirmed the presence of silver-containing nanoparticles in tissue from rats treated with ionic silver. These findings highlight that it is not entirely possible to distinguish between particle- and ion-dependent effects of silver nanoparticles, since, on the one hand, particles release ions and, on the other hand, ions form secondary nano-sized particles. This conversion between different silver species is significant for the interpretation of in vitro an in vivo studies and for risk assessment.

Keywords: Biokinetics/toxicokinetics, Exposure characterization, In silico modeling, In vivo toxicology, Metal/metal oxide nanomaterials

22. Genotoxicity Induced by Nanoparticles on Human Lymphoblastic Cells (TK6)

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The use of nanoparticles is increasing worldwide and there are many nanotech-based daily products available in the market. The toxicity of nanoparticles results from their extremely small size which can be transported easily into the blood stream and other organs. We aimed to study the genotoxicity of two nanoparticles, Titanium dioxide (TiO_2-NPs) and Zinc oxide (ZnO-NPs), in TK6 cells by micronucleus assay. The cells were tested at 8, 24, and 48 hours after exposed to 0.10, 0.25, 0.50 and 1.00 µg/mL of TiO_2-NPs particles size.

Keywords: Genotoxicity

23. Lipid-coated Magnetic Nanoparticles for Glioblastoma Chemo-sensitization

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Glioblastomas (GBMs) are an aggressive form of brain cancer with extremely poor patient prognoses attributed to chemotherapeutic drug resistance and challenges in delivering therapeutics to the brain. This presentation will describe our efforts to design multifunctional lipid-coated magnetic nanoparticles (L-MNPs) that are capable of imaging and treating GBMs through siRNA-mediated chemo-sensitization. L-MNPs are created using a dual solvent exchange method that yields monodispersed magnetite nanoparticles approximately 30-40 nm in diameter with tunable surface charge based on the ratio of cationic to polyethylene glycol (PEG) lipids in the coating. The L-MNPs exhibit low cytotoxicity, are effective magnetic resonance imaging (MRI) contrast agents, have high capacity for siRNA binding and intracellular delivery (transfection), achieve gene silencing (protein knockdown) associated with DNA cross-link repair, and can release siRNA passively or actively in the absence or presence of a radiofrequency electromagnetic field, respectively. All of these properties are sensitive to the cationic lipid:PEG-lipid ratio and the resulting PEG conformation (mushroom vs. brush).

Keywords: Biomedical/therapeutic applications, Metal/metal oxide nanomaterials, Physicochemical characterization

24. Dissolution of Silver Nanoparticles by an In Vitro Human Digestive Assay: Quantification of Free Ions/Ion-Complexes Bioavailability for Duodenal Absorption

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The agro-food market has experienced over the last years the revolution of nanotechnologies, exploiting and employing the well-known biocidal activity of silver nanoparticles (AgNPs) in packaging material as additives or to extend the shelf life of consumer products (Gaillet and Rouanet, 2015). Human exposure to AgNPs through ingestion (voluntary or accidental) is likely and therefore the characterization of the molecular species arising from biotransformation processes in human biological fluids (e.g. the simulating gastrointestinal juices) has toxicological importance. Outcomes of these studies may help the creation of proper models of risk assessment and improve the risk perception of stakeholders for AgNPs (Stone et al, 2014). Within this framework, we characterized the behavior and fate of AgNPs when in contact with synthetic human digestive juices, using complementary analytical techniques and following standardized procedures (i.e., SOPs developed under the EC project Nanoreg). We implemented a dynamic in vitro model to simulate the oral ingestion and the passage along the gastrointestinal tract, with salts, proteins composition, pH differences and transit times alike the in vivo digestion. Results show that AgNPs progressively aggregate along the digestive compartments and partially dissolve in ions. Ions were quantified and demonstrated to be in two different states, namely free or chelated to the digestive matrices. The ultrafiltration quantified and “speciated” such complexes showing that their bioavailability was different in the various digestive compartments. Therefore, the molecular species bioavailable for duodenal absorption are represented by a polydispersed matrix of low amount of primary AgNPs, agglomerates/aggregates, free ions and ion chelates.

Keywords: Toxicological mechanisms, Dissolution, free silver ions bioavailability, risk assessment

25. Direct Visualization of Nanoparticles in Tissues following Inhalation at Concentrations that are Relevant to Occupational Exposures

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Inhalation exposure to engineered nanomaterials may result in pulmonary and/or systemic health effects. The purpose of this study was to investigate the potential for biodistribution of industrially used metal oxide nanoparticles (silica, ceria, alumina) following inhalation at concentrations that are relevant to occupational exposures. A rat model of inhalation exposure (4-6 hours) at three concentrations was used, with lung and secondary organs harvested 24 hours or 7 days post-exposure, and prepared for direct visualization by brightfield (BF) and enhanced darkfield microscopy (EDFM). Corresponding BF and EDFM images were captured to provide histological information of physiologic relevance. EDFM revealed nanoparticles (NPs) in lung and ancillary tissues (lung lymph nodes) as well as major organs of excretion (liver, spleen, kidney). Imaging also revealed NPs within pulmonary blood vessels and localization within anatomical sub-structures of physiological relevance. Results suggest that metal oxide NPs used in occupational settings have the potential to biodistribute via the circulatory and lymphatic systems following inhalation exposure. The immediate next step in this investigation is to confirm the composition of the NPs in tissue samples through hyperspectral imaging (HSI) and mapping. This will set the stage for further development of EDFM and HSI to investigate the relative distribution of NPs throughout the body.

Keywords: Biodistribution, Metal/metal oxide nanomaterials, Occupational safety, darkfield microscopy

26. Evaluation of the Cytotoxicity Profile of Commercial Amorphous Silicon Dioxide Nanoparticles

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Silicon dioxide nanoparticles (SiNPs) are finding more use in products, increasing the likelihood of environmental and health impacts. The cytotoxic and pro-inflammatory potency of three amorphous SiNPs of similar size (5-15, 10-20, 12 nm) in three cell lines of distinct functional and species origin (human epithelial A549, human THP-1 and mouse J774A.1 macrophage) was investigated to examine the influence of physico-chemical and biological factors on the toxicity of SiNPs. Cellular LDH and ATP, BrdU incorporation, resazurin reduction and cytokine release were measured. SiNP (12 nm), the most cytotoxic and inflammogenic nanoparticles had the highest surface acidity and agglomerate size in dry form, the lowest trace metal and organic content, total surface area, and small agglomerate size in culture media. Surface acidity, a measure of particle polarity was the most significant determinant of the overall adverse biological activity of SiNPs. Mouse macrophages were more sensitive than human macrophages or epithelial cells. Correlation analysis revealed congruence in the cytotoxic response of individual cell lines. The cytokine responses differed across cell lines, owing to differential expression from the cell types, also corroborated by cell type-specific differences in inflammation-associated cellular pathways. Integration of the cytotoxic and inflammatory potency estimates allowed for a comprehensive determination of the biological reactivity of the SiNPs in cells, combined with a thorough characterization of SiNPs. The approach shows promise as a useful tool for first-tier screening of particle toxicity towards more reliable safety assessments of SiNPs. Funding provided by the Chemicals Management Plan, Nanotechnology Fund, Health Canada.

Keywords: Alternative testing methods/strategies, Hazard ranking/characterization, In vitro toxicology, Physicochemical characterization

27. Diffusivity and Biodistribution of PEGylated Single Wall Carbon Nanotubes in Rodent Brain

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The advent of nanotechnology has enabled the creation of nanoscale materials with unique chemical and physical properties, evoking the interest of the biomedical field due to the potential capacity of these small structures of crossing biological membranes, moving throught live tissue, biocompatibility, low immunogenicity and for their high stability in physiological media. The aim of this study is to evaluate the biodistribution and the diffusion of PEGylated Single Wall Carbon Nanotubes (SWCNT-PEG) in rodent brain. A DNA oligonucleotide ds (GT)15 conjugated with a 5 kDa molecular weight PEG wrapped SWCNT has been used for the diffusitvity study and a commercial sample of SWCNT-PEG (PEG Mw=600 Da) in stable dispersions for the biodistribution test. The biodistribution experiment was performed by intravenous administration of 0.05, 0.25 and 1.25 mg/kg of SWCNT-PEG dispersion in Wistar rats and tissues were removed after 24h of exposure for analysis. The diffusivity test was performed with mouse brain slices exposed to different concentration of PEG-(GT)15 - SWCNT in different time points. All protocols were approved by the Institutional Committee on Animal Care (process number 0514-039-17 - MIT/USA and 006/2014 – FURG/Brazil). Tissues were analyzed by Raman spectroscopy for both tests. This preliminary data suggests that PEGylated SWCNTs are able to reach and diffuse throught the brain tissue without causing a severe local reaction and tissue damage. Therefore, they may have the potential for several biomedical and nanobiotechnological future applications. Funding provided by NIH NIEHS Nanosafety Consortium (USA); CsF (CNPq), CAPES, Nanotoxicology Network, INCT – Carbon Nanomaterials (Brazil).

Keywords: Biomedical/therapeutic applications, Carbon-based nanomaterials

28. Immunotoxic and Immunomodulatory Effects of Subchronic Inhalation Exposure to Zinc Oxide Nanoparticles on Splenocytes in Mice

Táňa Brzicová[1], Eliška Javorková[1], Alena Zajícová[1], Vladimír Holáň[1], Zbyněk Večeřa[2], Omar Šerý[3], Kristýna Vrbová[1], Jan Topinka[1], Pavel Rössner[1]. [1]Institute of Environmental Medicine, Czech Academy of Sciences; [2]Institute of Analytical Chemistry, Czech Academy of Sciences; [3]Faculty of Science, Msaryk University, Czech Republic.

To evaluate potential immunotoxic and immunomodulatory effects of zinc oxide nanoparticles (ZnO-NPs), spleen cells from mice after subchronic (90-day) whole body inhalation exposure were isolated. Concentrations of ZnO-NPs (geometric mean diameter <10 nm) were 6×10^4 particles/cm^3 and 2×10^6 particles/cm^3, corresponding to 20 and 625 μg/m^3 respectively. Mice exposed to filtered air served as a control group. Spleens of the exposed mice did not exhibit any signs of pathology and no difference in spleen size between mouse groups was observed. Analyses of zinc content in organs are ongoing. Alterations in composition of spleen cell populations were evaluated by phenotyping different immune cell subtypes by flow cytometry. The proportions of T-lymphocytes and their subpopulations (CD3+, CD4+, CD8+) and B-lymphocytes (CD19+) in fresh splenocytes did not differ between exposed and control mice. However, a significant decrease in granulocytes in exposed mice was observed, namely in eosinophils (CD11b+ CD193+) and neutrophils (CD11b+ Gr1+). Similarly, monocyte counts were reduced in spleens from both higher and lower dose exposed mice. Using ELISA, production of selected cytokines (pro-inflammatory IL-2, IL-4, IL-6, IL-17, IFN-γ, and anti-inflammatory IL-10) was measured in non-stimulated and lipopolysaccharide- or concanavalin A-stimulated cells. Overall, ZnO-NPs did not significantly affect cytokine concentrations. The results suggest that subchronic inhalation exposure of mice to Zn-ONPs in concentrations of 20 and 625 μg/m^3 did not cause profound changes in immune response. However, slightly decreased neutrophils and myeloid cell populations may indicate potential immunosuppressive effects of ZnO-NPs. Funding provided by Ministry of Education Youth and Sports CR (#LO1508).

Keywords: In vivo toxicology, zinc oxide nanoparticles, whole body inhalation, immunotoxicity

29. Comparative Profiling of In Vivo Neuroinflammation from Intracranial Administration of Functionalised Carbon Nanotubes and Graphene Oxide

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Carbon nanomaterials, such as graphene-based materials, have been recently proposed as suitable for brain implant coatings due to their lack of unwanted side-effects on neuronal function upon interaction. This raises the interest to assess whether these materials are inert as platforms for brain applications. In particular, a critical safety parameter regarding the use of nanomaterials in the brain is the induction of inflammatory responses. To this end, a variety of nanosystems were injected into the striatum of mouse brains and their response was evaluated at different time points. The data revealed that different nanosystems elicited different patterns of inflammation in the injected area. Lipid-based nanomaterials increased inflammatory marker expression levels, peaking at day 2 and persisting up to day 7 after injection, more notably for cationic liposomes compared to negatively surface charged ones. Administration of carbon nanotubes (oxidised or aminated) or graphene oxide elicited an upregulation of pro-inflammatory markers only at day 2, reaching a level comparable to the negative control after one week. While monitoring the diffusion of the inflammatory response around the site of injection, we found a persistent and significant pro-inflammatory response for cationic liposomes whereas administration of carbon nanomaterials revealed a similar trend to the negative control at all time-points. The present findings point out a sustained inflammatory response after administration of liposomes regardless of their surface charge characteristics, while carbon-nanomaterials, especially graphene oxide, seemed well-tolerated by the brain parenchyma. These observations suggest that carbon nanomaterials can be safely considered as non-inflammogenic materials in brain applications.

Keywords: Biomedical/therapeutic applications, Carbon-based nanomaterials, Emerging nanomaterials, In vivo toxicology

30. Coating Hydrophobicity Modulates the Intestinal Absorption of Nanomaterials

Joan Cabellos, Socorro Vázquez-Campos, Camilla Delpivo. Leitat Technological Center, Spain.

The systemic effects of nanomaterials after oral exposure will greatly depend on the degree of absorption. Understanding the role of nanoparticle coatings in the absorption process is essential to facilitate read-across among different nanoparticles, or even among different life cycle stages of a nanoparticle. The translocation of CeO_2 nanoparticles (4-8 nm; provided by PlasmaChem), uncoated and with coatings of different hydrophobicity (citrate, a polyethylene glycol derivative, and dodecylphosphonic acid) was evaluated in the Caco-2 monolayer model by quantifying the amount of Ce2+ in the basal compartment by ICP-MS. In addition, a fourteen-day repeated oral administration study in Sprague-Dawley rats was performed with CeO_2-citrate and CeO_2-PEG nanoparticles at 120 mg/kg (dose expressed as CeO_2). Ce2+ levels were quantified by ICP-MS in several tissues (small intestine, Peyer's patches, mesenteric lymph nodes, caecum, liver, spleen, kidneys, epididymis, and lungs). In addition, the presence of CeO_2 nanoparticles in sections of small intestine (with and without Peyer's patches), spleen, and liver was investigated by TEM. In vitro translocation was below detection limits in all cases, regardless of the coating type. In contrast, an increase of Ce2+ levels in tissues of the exposed rats was recorded. These levels were around 2-fold higher in liver, kidney and spleen of rats that had received CeO_2-citrate nanoparticles than in those receiving CeO_2-PEG nanoparticles ($p < 0.05$). TEM micrographs showed the presence of nanoparticles in some sections of the small intestine and the spleen of CeO_2-PEG exposed animals.
Funding provided by the EU FP-7 GUIDEnano, Grant Agreement No. 604387.

Keywords: Biodistribution, Biokinetics/toxicokinetics, Cerium oxide nanoparticles, In vitro toxicology, In vivo toxicology, Metal/metal oxide nanomaterials

31. Influence of Different Experimental Variables in the Prediction of Nanomaterial Intestinal Absorption

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Intestinal absorption is a key determinant of nanomaterial systemic toxicity after oral exposure. The Caco-2 monolayer model is widely used to predict the permeability of substances across the intestinal barrier. The inclusion of M-cells should improve the relevance of this model for nanomaterials, since transcytosis through M-cells is considered to play a major role in the intestinal absorption of particles. A Caco-2/Raji B coculture was established to induce the transformation of enterocytes into M-cells, which were characterized by immunocytochemistry and SEM. The permeation of TiO_2 nanoparticles was evaluated in the Caco-2 and in the Caco-2/M-cell models. In each of them, we evaluated two nanomaterial dispersion methods as an additional variable that could influence nanomaterial translocation. Experimental variables are also expected to influence nanomaterial absorption in in vivo models. The effect of fasting on TiO_2 nanoparticle absorption was evaluated in a fourteen-day repeated oral administration study in Sprague-Dawley male rats. At the end of the administration period, clinical signs of toxicity and gross pathology were assessed, and the presence of nanomaterials in several tissues was evaluated by ICP-MS and TEM. The levels of Ti in the basal compartment were above the detection limit only when M-cells were present in the model. Slight differences were found between the two dispersion methods, possibly due to the fact that they resulted on different aggregation sizes. Some absorption did occur in the in vivo study, but only minor differences between fasted and unfasted conditions were found. Funding provided by Funded by EU FP-7 GUIDEnano, Grant Agreement No. 604387.

Keywords: Biodistribution, Biokinetics/toxicokinetics, In vitro toxicology, In vivo toxicology, Metal/metal oxide nanomaterials

32. Bio-nano Interface Models Applied to the Investigation of Nanoparticles Cell Uptake: Proof of Concept using Real Membrane Models

Juliana Cancino-Bernardi, Paula M. P. Lins, Valeria S. Marangoni, Valtencir Zucolotto. University of São Paulo, Brazil.

Nanoparticle-based materials have proven to be excellent platforms for biomedical applications. However, the understandings on the interactions occurring at the nano-bio interfaces are still a challenge. In a tentative to overcome these challenges, we report here an in situ study on the interactions occurring between natural cell membranes -from health and cancer cells - and gold nanoparticles and nanorods. The membranes were isolated from the original cells and reconstructed as Langmuir monolayers at the air/water interface. The difference in the lipidic composition of the extract membranes was investigated by thin-layer chromatography with flame ionization detection. The influence of the morphology and surface charge of gold nanorods and nanospheres on the structure, surface pressure, molecular area, compressibility, and phase-transition of the membranes were evaluated. Our results revealed that the uptake of nanoparticles through cell membrane is affected by the lipidic composition of each cell line. Moreover, we show that the surface charge of nanoparticles plays a significant role in their ability to interact with cell membranes (results corroborated with in vivo and in vitro assays). Our study pointed to the importance of combining complementary techniques and models to better understand the nanotoxicity of nanomaterials. Funding provided by Fapesp 2012/03570-0. Knowledment to CAPES and CNPq - Brazil.

Keywords: Alternative testing methods/strategies, Methods, Systems biology/toxicology, Toxicological mechanisms

33. DNA Damage Induced by Long-term Exposure of A549 cells to Titanium DioxideNanoparticles

Marie Carrier[1], Lucie Armand[1], Adeline Tarantini[1], Karin Pernet-Gallay[2], Caroline Desvergne[1], Iseult Lynch[3], Thierry Rabilloud[4], Helene Diemer[5], Jean-Marc Strub[5], Sarah Cianferani[5], Alain Van Dorsselaer[5], Nathalie Herlin[1]. [1]CEA; [2]INSERM; [4]CNRS, [5]University of Strasbourg, France; [3]University of Birmingham, UK.

Up to now in vitro nanoparticle toxicology has been restricted to acute exposure regimens, using cell lines exposed to high concentration of NPs for short times, in submerged conditions. In an attempt to develop more realistic in vitro exposure scenarios, we evaluated cell response to chronic exposure to TiO_2-NPs. To achieve this goal we continuously subcultured A549 cells, for up to 2 months, in culture medium containing 0, 1, 2.5, 5, 10 or 50 µg/mL TiO_2-NPs. The impact of this exposure regimen was assessed after 24 h, 1 week, 2 week, 1 month and 2 month of cells exposure, with a special focus on DNA damage. Using comet assay, 53BP1 immunostaining, micronucleus assay, we show that chronic exposure to TiO_2-NPs induces oxidative damage to DNA. The intensity of damage increases with exposure time, with maximal impact after 1 month of exposure. Proteomics insight gives clues on the mechanisms underlying this damage. Finally, post-exposure of these chronically exposed cells to sublethal concentrations of methyl-methane sulfonate induces increased cyto- and genotoxicity. Taken together, our results show that chronic exposure to TiO_2-NPs causes genotoxic damage to A549 cells and may sensitize them to other genotoxic agents. Funding provided by CEA (Nanoscience and Toxicology programs), by the European Commission's 7th Framework Programme (NanoMILE, NMP4-LA-2013-310451) and received support from QualityNano Project (European Community Research Infrastructures, INFRA-2010-262163), and its partner University of Birmingham. It is a contribution to Labex Serenade (ANR-11-LABX-0064) funded by the "Investissements d'Avenir" French Government program through A*MIDEX project (ANR-11-IDEX-0001-02).

Keywords: Genotoxicity, In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

34. Endoplasmic Reticulum Stress is an Earlier Biomarker for Nanotoxicological Evaluation

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Adverse response pathways for adverse health effects evaluation are regarded as the preferred toxicity testing strategy in the 21st century. In vitro AOP pathway test is a sensitive method to give hazard identification ahead of realistic toxic effects formation. Endoplasmic reticulum (ER) stress pathway, also known as unfolded protein response (UPR), is a conserved cellular self-protection mechanism for monitoring the steady state of the cell functions. It happens quickly after the concentration of outer exogenous stimulators exceeds the physiological threshold of cellular self-protection, and prolonged stress activates apoptotic cell death pathways. It had been reported that free gadolinium ions lead toxicity through inducing ER stress response pathway. Herein, by using mouse model, we parallel compared their toxicity and ER stress-inducing ability of three T1 MRI contrast agents including clinically used GBCA, synthesized ESIONs and MnO NPs at their MRI application dosages. From our results, ESIONs show higher safety and more favorable property for potential clinical applications if compared to manganese oxide and clinically used GBCA (Gadopentetate Dimeglumine). Our results illustrate there is an exciting improvement on the health safety and clinical therapeutic benefits with the development of well characterized nanomaterials. Both in vitro and in vivo experiments on other nanomaterials, such as AgNPs, ZnO NPs, TiO_2 NPs, etc., demonstrated that endoplasmic reticulum stress is an earlier biomarker for nanotoxicological evaluation.

Keywords: Adverse outcomes pathway analysis

35. Biotinylated Chitosan Surface Modified PLGA Nanoparticle: Preparation, Stability, In Vivo Toxicity and Pharmacokinetics

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Based on our previous work on the poly(D,L-lactide-co-glycolide) nanoparticles modified with biotinylated Chisosan (Bio-CS-PLGA NPs), we further studied the stability and toxicity of Bio-CS-PLGA NPs, and the sustained release behavior in vivo of drug loading in NPs. The size of Bio-CS-PLGA NPs was 214.4±21.0 nm determined by the laser light scattering technique, and their morphology were spherical characterized by scanning electron microscopy (SEM). The storage stability of Bio-CS-PLGA NPs was observed in distilled water. Bio-CS-PLGA NPs were stored for at least 4 weeks with no significant size and ζ potential changes. The safety of Bio-CS-PLGA NPs was studied through single dose toxicity test in mice, and the result showed that NPs were well tolerated at the dose of 200 mg/kg in mice. Epirubicin (EPI)-loaded Bio-CS-PLGA NPs were also prepared and characterized in this study. Moreover, the in vivo pharmacokinetics of Bio-CS-PLGA NPs was investigated. Compared with the free EPI group, the NPs group exhibited higher plasma drug concentration, longer half-life time ($t_{1/2}$) and the larger area under the curve (AUC). All results suggested that Bio-CS-PLGA NPs were stable, safe, and showed a promising potential on improving the bioavailability of the loaded drug of the encapsulated drug.

Keywords: Biokinetics/toxicokinetics

36. Biological Surface Adsorption Index: Environmental Applications and parallel Molecular Dynamics Simulations

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Quantitative analysis of the interactions between nanomaterials and environmental contaminants (e.g. pesticides) in natural water systems is crucial for the development of nanomaterial-based tools for the detection of potential toxins, and designing effective strategies for environmental remediation. Previously the Biological Surface Adsorption Index (BSAI) has demonstrated promising capabilities of characterizing and predicting small organic molecule nanoparticle interactions based on experimental data. In this study, we report its application toward environmental endpoints by analyzing the interactions of a select group of nanomaterials with a variety of pesticides. Statistical modeling was conducted on the experimental adsorption data using polynomial BSAI models and artificial neural network methodologies. Finally, clustering analyses were performed on these results to categorize the nanomaterials based on surface physicochemical properties. These quantitative approaches using the experimental data support the application of BSAI modeling in environmental contaminant detection and remediation. In parallel, n parallel, explicit-solvent molecular dynamics was applied to evaluate the ability of selected MD models of carbon nanotubes to predict adsorption coefficients for the small organic molecules used in the BSAI experiments. The results are directly compared to those derived from experimental measurements. The calculations are highly predictive of the relative adsorption affinities, with excellent correlation between calculated and measured values of the adsorption coefficients, suggesting under certain conditions, pure computational MD models may be used for model building and predictive purposes without experimental data, provided that sound MD models exist for the underlying nanomaterial.

Keywords: In silico modeling, Physicochemical characterization, Adsorption, Environmental remediation

37. High Occupational Health Risks of the Airborne Nanoparticle Aerosol Emitted from Electrical Discharge Machining Process

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Electrical discharge machining (EDM) is widely used for manufacturing desired shapes of hardened metal workpieces. Airborne exposure to nanoscale and respirable particles were investigated with regard to the aerosol characteristics of an EDM workshop. The total number concentration of the aerosol was multimodal, with the highest peak maxima during the working hours of 10:00 am and 3:00 pm. The majority of the released particles were smaller than d = 100 nm, with the maximum amount sized 40 nm. A large quantity of metallic elements, including Fe, Al and Cu, were found in the aerosol particulates emitted from EDM processing. Furthermore, the aerosol particles exhibited higher cellular toxicity and ROS producing ability in human alveolar epithelial cells (16HBE) when compared to the atmospheric background. Our results indicate substantial hazards arising from exposure to polluted atmosphere of the EDM workshop. Effective exposure controls and protections are thus strongly recommended. Our findings emphasize the requirement of a better pre-design of metal workshops and associated effective exposure controls including ventilation, area, temperature, labor safety equipment. For such purpose, more detailed, complete and full studies are needed to analyze the EDM airborne pollution. We acknowledge the financial support from Natural Science Foundation of China (21477029 and 21277080) and the European Commission through the Seventh Framework Programme for Research and Technological Development (FP7-MARINA; Grant agreement 263215).

Keywords: Environmental nanotoxicology, Exposure assessment, Occupational safety, Risk assessment

38. Brain-targeting of Proteins and Peptides by Nano-based Delivery System

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Growing amounts of proteins and peptides have been shown beneficial for central nervous system diseases such as tumors, Alzheimer’s disease, and Parkinson’s disease. However, the existence of blood-brain barrier (BBB), the instability and poor bioavailability of proteins and peptides in vivo are still challenging the application of these therapeutic molecules. Nanotechnology is one of the most studied strategies for delivering drugs to brain. Here, we reviewed recent progress of delivering therapeutic proteins and peptides to brain by using nanotechnology. Nanoparticles based with different carriers such as liposomes, PLA, PLGA, PBCA and PCL were utilized to transport proteins or peptides to brain. Nanoparticles modified with specific brain-targeting molecules including cell penetrating peptides, some ligands, some antibodies and other “Trojan Horse” molecules would increase their accumulation in brain. Besides, inorganic nanomaterials are getting more and more attention in delivering drugs for their big specific surface area, shape and size controllable, and unique electric, optical, thermodynamic and catalytic properties. Nanocarriers which construction of inorganic and organic nanomaterials are also been studied for brain targeted delivery system. In conclusion, with the development of nanotechnology and biotechnology, the nano-based delivery system which have properties of good biocompatibility, reliability, high entrapment efficiency, and specific brain-targeted for therapeutic proteins and peptides would be development soon.

Keywords: Biomedical/therapeutic applications, Green nanomaterials, Methods

39. Long-Fibre Carbon Nanotubes Induce Pleural Mesothelioma Recapitulating Human Disease

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Exposure to asbestos fibres causes pathological changes including malignant mesothelioma. Length-dependent retention of fibres in the pleural cavity and chronic inflammation play a key role in carcinogenesis. Engineered carbon nanotubes (CNT) have similar to asbestos high aspect-ratio and thus may pose an asbestos-like inhalation hazard, however the molecular mechanisms underlying CNT carcinogenic potential have not been sufficiently explored. Using a mouse model of direct injection of short and long asbestos fibres and short and long CNT into the pleural cavity, we compared molecular the changes in the pleura over prolonged exposure times following injection. We show a common molecular signature in response to long CNT and long asbestos throughout disease progression. Key molecular events encompass changes in gene expression and pro-oncogenic signaling pathway activation, oxidative DNA damage, and increased mitosis and proliferation.

Instillation of long CNT into the pleural cavity of mice induces chronic inflammation and pro-oncogenic changes leading to development of malignant pleural mesothelioma. Long CNT-induced mouse tumours recapitulate molecular features of asbestos-induced human disease, including disruption of the tumour

suppressor genes CDKN2A and NF2, “gatekeepers” in mesothelioma development. Epigenetic changes

induced by pathogenic fibres occur at the pre-neoplastic stage and play a key role in the progression of pleural inflammatory lesions to malignant mesothelioma. Together, these data highlight commonality in the hazard mechanism of long pathogenic fibres at the molecular level. Crucially, our findings reinforce concerns that long CNT may pose an asbestos-like hazard, leading to malignant mesothelioma. Funding provided by Medical Research Council (MRC).

Keywords: Carcinogenicity, Carcinogenicity, Carbon-based Nanomaterials, In vivo Toxicology, Toxicological Mechanisms

40. Titanium Dioxide-induced Gene Expression Profile in Rat Lung, a Sub-acute Inhalation Study

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Due to the growing use of nanoparticles in industrial processes, the number of workers potentially exposed is increasing while the toxicological properties of these compounds are not fully known. Inhalation represents the main route of occupational exposure. In this respect, the experimental toxicology studies conducted by inhalation in animals appear to be the most relevant for the early evaluation of the hazard associated with exposure to nanoaerosols. In this work we study the pulmonary toxicological properties of titanium dioxide using conventional and molecular toxicological approaches. For this, we exposed Fischer 344 rats by nose-only inhalation 6 hours/day, 5 days/week for 4 weeks to a nanoaerosol of TiO_2 (10 mg/m3). Lung samples have been collected up to 180 days after the end of exposure. Biochemical and cytological analyses of the broncho-alveolar lavage fluid (BALF) showed a strong inflammatory response up to 3 days which decreased overtime but persisted 180 days after exposure. In addition, a gene expression profiling experiment showed an overexpression of genes involved in inflammation that persisted in the chronic phase. These observations are consistent with physiopathological changes. Moreover, this experimental approach revealed also an overexpression of genes involved in vasodilatation and oxidative stress. To complete these data, proteins expression will be assessed using western blot or ELISA, as well as proteomics of BALF. These analyses will enrich the transcriptomic and cytological data. Finally, analysis of long-term epigenetic modifications (DNA methylation, acetylation) will be performed to observe possible chronic gene expression modification, involved in physio-pathological processes.

Keywords: High throughput screening, In vivo toxicology, Risk assessment, Toxicological mechanisms

41. Studies on Toxicological Assessment of Tungsten Oxide Nanoparticles and Microparticles in Female Wistar Rats after Acute Oral Exposure

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Tungsten oxide nanoparticles (WO_3 NPs) are being explored in various fields. Limited toxicological data on WO_3 NPs makes it difficult to determine if there is a risk associated with these NPs. Hence, the aim of this study was to investigate the toxicity of nano and micron sized WO_3 after acute oral administration in female Wistar rats. The particles were characterized utilizing TEM, DLS and LDV. The genotoxicity studies were conducted using micronucleus test, comet, chromosomal aberration assays. Biochemical and histopathological parameters as well as biodistribution patterns were also evaluated. The characterization of WO_3 NPs and MPs by TEM was 53.2±1.91 nm and 5.17±3.18 μm. The results revealed a significant increase in DNA damage in peripheral blood leucocytes, liver and micronuclei in bone marrow after exposure to 1000 mg/kg bw/day dose of WO_3 NPs. Significant alterations in AST, ALT, GSH, CAT and MDA levels in serum and liver at the highest dose of WO_3 NPs. W biodistribution was observed in all the tissues in a dose, time and organ dependent manner. The maximum amount of W was found in the liver and least in the brain of the treated rats. More of the W was excreted in the feces than in the urine. Histopathological examination did not reveal any alterations at any dose levels with both particles. These findings provide initial data of the probable genotoxicity and biodistribution of NPs and MPs of WO_3 generated through acute oral treatment. This work was supported by AOARD, Japan (FA2386-11-1-4085).

Keywords: Biodistribution, Exposure assessment, Genotoxicity, In vivo toxicology

42. In Vitro Assessment of Size, Surface Chemistry and Protein Corona Effects on Hepatic Uptake and Toxicity of Gold Nanoparticles in Human Hepatic Cells

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Gold nanoparticles (AuNP) have been used in biomedical and industrial applications as well as in cosmetic products. The liver is a major organ for AuNP accumulation. We investigated the hepatic uptake of 5µg/mL of 40/80nm AuNP with branched polyethylenimine (BPEI), lipoic acid (LA) and polyethylene glycol (PEG) with or without plasma or human serum albumin (HSA) in human hepatocytes (HC) at 0.25, 0.5, 1, 3, 6, 12 and 24h with ICP-MS. Cell uptake increased up to 24h except for BPEI-AuNP with plasma or HSA. At 24h, rank order of uptake was 80nm BPEI-AuNP > 40nm BPEI-AuNP > 80nm LA-AuNP > 40nm LA-AuNP > 80nm PEG-AuNP ≥ 40nm PEG-AuNP. Plasma and HSA inhibited uptake of 40/80 nm BPEI-AuNP and 40nm LA-AuNP but increased with 80nm LA-AuNP. At 24h, all AuNP with or without plasma were nontoxic except for 40/80nm BPEI-AuNP (24h LC50 values, 169.1 and 175.1µg/mL, respectively). BPEI-AuNP significantly induced reactive oxygen/nitrogen species at over 150µg/mL up to 24h. BPEI-AuNP reduced CYP3A4 activity in HC (24h IC50 values, 118.7 and 108µg/mL); for CYP1A2 119.9 and 156.2µg/mL, respectively. This research suggests that protein coronas modulate cellular uptake of AuNP and its surface charge. AuNP-induced oxidative stress, cytotoxicity and loss of cytochrome P450 activity may contribute to liver toxicity in humans. (Supported by the Nanotechnology Innovation Center of Kansas State).

Keywords: Biocorona, Biomedical/therapeutic applications, In vitro toxicology, Metal/metal oxide anomaterials

43. Crossover between Anti- and Pro- Oxidant Activities of Graphene Quantum Dots in the Absence or Presence of Light

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Graphene Quantum Dots (GQDs), as a new class of zero dimensional fluorescent carbon materials, hold the promise for broad applications in various fields of biomedicine and optoelectronics. In this study we report that GQDs can protect cells against oxidative damage, which is attributable to the efficient scavenging of reactive oxygen species (ROS) and nitrogen-centered free radical evidenced using electron spin resonance (ESR) techniques. On the other hand, upon exposure to blue light, GQDs enhance intracellular ROS levels and reduce cell viability, exhibiting significant phototoxicity. For the first time, ESR was employed to systemically reveal the mechanism for generation of a series of free radicals during photoexcitation of GQDs. We propose that singlet oxygen is generated by photoexcited GQDs via energy transfer and electron transfer pathways. Hydroxyl radicals and superoxide radical anions result from band-to-band transition and creation of electron-hole pair, respectively reacting with surrounding molecular oxygen or H_2O. Additionally, we found upon photoexcitation, GQDs accelerated the oxidation of nonenzymic antioxidants including ascorbate and glutathione, and promoted lipid peroxidation, reactions possibly related to the phototoxicity of GQDs. This study demonstrates the antioxidant activities of GQDs and pro-oxidant activities upon light irradiation. A deeper understanding of the dual properties of GQDs will help better define conditions for appropriate use of GQDs for photodynamic, photocatalytic or antioxidant applications.

Keywords: Carbon-based nanomaterials, In vitro toxicology, Physicochemical characterization, Toxicological mechanisms

44. Biological Impact of Sub-chronic Repeated Exposures to Aerosolised MWCNTs on Healthy and Asthmatic Lung Cells at Occupational Relevant Doses

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The extraordinary properties of carbon nanotubes (CNTs) have been used to a plethora of commercial applications, leading to a massive increase in their production rate. Thus, occupational human exposure via inhalation is highly possible during either their production and/or disposal. Despite increased investigation into the pulmonary toxicity of CNTs, so far most studies have focused only on acute/short-term exposures, with knowledge of the chronic effects of such exposures currently limited. The aim of this study therefore, was to mimic the inhalation of multi-walled CNTs (MWCNTs), using the Air-Liquid Interface Cell Exposure system (ALICE) for aerosol exposures to deposit MWCNTs onto primary bronchial cells (MucilAir TM) derived from healthy donors and donors with asthma pathology, at realistic doses that represent human occupational lifetime exposure (10µg/cm² for 5 weeks of repeated exposures/5 days per week). No cytotoxic reactions or morphological changes were observed. Chronic CNT exposure did though induce an increased pro-inflammatory and oxidative stress response in both healthy and asthmatic cells. The latter revealing stronger and more durable long-term effects compared to healthy cells, indicating that individuals with asthma may be more prone to adverse effects from MWCNT exposure compared to non-asthmatic populations. This study is a major step further to most in vitro toxicity studies as it was possible not only to realistically mimic full working lifetime exposure to MWCNTs for the first time, but also to investigate differences in adverse effects between normal and diseased lung cells in response to CNTs at concentrations relevant to occupational exposures.

Keywords: Alternative testing methods/strategies, Carbon-based nanomaterials, In vitro toxicology, Risk assessment, Sub-chronic exposures, occupational exposures

45. Development of a Nano-enabled Bio-packaging for the Cosmetic Packaging Industry- Toxicological Aspects to Meet Regulatory Needs

Mona Connolly, Helinor Johnston, Teresa Fernandes, Vicki Stone. NanoSafety Research Group, Heriot-Watt University, UK.

Nanotechnology is having an impact on many facets of life and revolutionising many industries, including the packaging industry. Nanoclays are being incorporated into bio-based polymers to create new packaging materials (e.g. for food, and cosmetics) with improved strength and barrier properties. Such nano-enabled packaging has huge market potential as nearly everything we use in our daily life comes packaged. It also represents a commercially viable and biodegradable alternative to the use of petroleum based plastics. Within the cosmetic industry such a biopackaging for organic cosmetic lines will allow the industry to fully differentiate its product offering and meet the demands of a truly organic and green product. The European FP7 funded project BioBeauty's aim is to develop such a packaging solution whilst ensuring consumer safety. According to cosmetic regulations "a cosmetic product made available on the market shall be safe for human health including the presence of small quantities of substances which may migrate from the packaging" (Cosmetic Regulation (EC) No 1223/2009 (Art.17)). In this study the safety of nanoclays was assessed using an integrated approach on testing and assessment (IATA), taking into consideration the physico-chemical properties of the nanoclays, existing toxicological data and a weight of evidence analysis to determine if additional testing was required. Testing was performed using non-animal in vitro alternatives for skin corrosion/irritation assessment and tests addressing events along the adverse outcome pathway (AOP) for skin sensitisation. This toxicological information, together with an exposure assessment (migration analysis) provided important for regulatory decision makers and risk assessors.

Keywords: Adverse outcomes pathway analysis, Alternative testing methods/strategies, Composite nanomaterials, Nanomaterial release

46. Bio-nanocomposites: Ensuring the Safe and Sustainable Development of These Next Generation Materials in the Packaging Industry.

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The use of bio-nanocomposites in the packaging industry has the potential to enhance biodegradability and remove one of the world's biggest sources of plastic waste. Specifically, the incorporation of nanoclays into natural and biodegradable polymers such as poly-lactic acid (PLA) enhances barrier properties and thus imparts significant improvements to PLA's performance as a bio-packaging solution. Within the European FP7 funded project BioBeauty, a bio-packaging is being developed for the cosmetic industry using PLA and sodium montmorillonite nanoclays organically modified with quaternary ammonium cations (organoclays). To ensure safety and to meet regulatory requirements any potential adverse effects associated with the use of organoclays were assessed. Nanoclays were screened for their skin corrosion and irritation potential using the HaCaT skin keratinocyte cell line and results were validated using the reconstructed human EpiDermTM skin model (OECD TG439). The response (cytotoxicity, cytokine production) of the J774 alveolar macrophage cell line was also assessed. Unmodified nanoclays did not produce any skin corrosion/irritation (no cytotoxicity (IC_{50}> 31.25 µg/cm^2) or pro-inflammatory cytokine production observed) in either test system. In contrast, organic modification significantly increased the skin irritation potential of the nanoclays (IC_{50} ranged from 3.4-17.8 µg/cm^2 in HaCaT cells depending on the modifier used) and increased pro-inflammatory cytokine (IL-18, IL-1α) production in the EpiDermTM skin model. Organoclays also exhibited higher cytotoxicity to macrophages. Thus the use of organically modified nanoclays in bio-nanocomposites may represent an increased occupational and consumer hazard. The obtained data will inform nanoclay selection for incorporation into new packaging to ensure their safe development.

Keywords: Composite nanomaterials, In vitro toxicology, Occupational safety, organically modified nanoclays

47. Pulmonary Toxicity and Genotoxicity of Carbon Nanotubes in Rats, a Subacute Inhalation Study

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Due to their physical and chemical properties, carbon nanotubes (CNTs) are among the most promising nanomaterials in terms of industrial use. In order to assess their toxicological properties, inhalation experiments performed in laboratory rodents remain the most suitable and reliable approach. We have performed sub-acute inhalation experiments in female Sprague Dawley with two CNTs: the “long and thick” NM-401 and the “short and thin” NM-403. Animals were exposed in nose-only chambers to these aerosols 6 hours/day, 5 days/week for 4 weeks. CNT aerosols were generated at a concentration of 0.5 and 1.5 mg/m^3 using an acoustic generator. Aerosols were fully characterized in terms of mass- and number-concentration; number- and mass-size distribution as well as morphology. Tissues were collected 3, 30, 90 and 180 days after the end of the exposure period. At the highest dose, both CNTs induced pulmonary inflammatory response 3 days after the end of exposure, this was demonstrated by an important neutrophilia in the broncho-alveolar lavage fluid (BALF) which however decreased overtime. Despite the presence of NM-401 within the lung, no significant histopathological changes were found; for NM-403 samples are still under evaluation. The genotoxicity of these nanomaterials was also assessed using the multiple-organ comet assay. Despite the presence of inflammation, no increase in DNA damage in lung or BALF cells as well as in liver, spleen or leukocytes was found. Additional experiments including cytokines expression are underway and may help to determine whether these two CNTs with different length and diameter may have distinct toxicological profiles.

Keywords: Carbon-based nanomaterials, Genotoxicity, In vivo toxicology

48. Potential Toxicity of Vanadium Metallic Debris and Vanadium Pentoxide

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Metallic materials, such as Ti-6Al-4V alloys, are commonly used as biomaterial in dental and orthopedic implants. In the body, they may suffer mechanical wear and electrochemical corrosion (tribocorrosion), leading to the release of metallic-based compounds or debris. Consequently toxicological potential effects of its alloying elements must be evaluated. Specifically regarding vanadium, there are few reports about possible toxicity of this element and its compounds released from metallic implants. Vanadium may induce ambiguous cellular responses and its toxicity is mainly dependent on its oxidation state and dose. Considering that vanadium may suffer oxidation into the human body, initial tests of potential toxicity of commercial vanadium pentoxide, presenting vanadium in its most common oxidation state (+V), were evaluated in vitro using mouse fibroblasts (NIH3T3 line). Different concentrations (from 0.2 μg/ml to 2,000 μg/ml) were tested in three different periods (24h, 48h and 72h) and evaluated by MTT and Crystal Violet assays. Initial results show no statistic difference for the lower tested concentrations in cell viability in comparison with the control group after 24h. However, a significant decrease in live cells was observed for all concentrations after 48h with complete cellular death for the two higher concentrations. The obtained results evidence the potential toxicity of vanadium to human cells. Additional in vitro tests are under progress against osseous and hepatic cells and the results will be compared with similar tests performed with Ti-6Al-4V debris obtained from tribocorrosion tests. Funding provided by CAPES, Fapesp.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Nanomaterial release, Toxicological mechanisms

49. Chemical Mechanical Planarization (CMP) of III-V Materials: Physiochemical properties, Behavior and Biological Impact of Pre- and Post-CMP Nanoparticle Slurries

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Chemical Mechanical Planarization (CMP) is a key enabling process used in semiconductor manufacturing to achieve local and global planarization of layers. CMP slurry typically contains fine abrasives (alumina, ceria or silica NPs) along with oxidizers and additives. In 2014, about 2.4 million metric tons of silica NPs was used by the semiconductor industry. In the pursuit to develop faster electronics, the semiconductor industry is poised to use large volumes of CMP slurries made up of colloidal silica (c-Si) NPs to polish toxic III-V materials such as GaAs and InP. In spite of such large-scale use, little is known about their fate, behavior and biological impact. Particularly, the physiochemical properties and biological effect of c-Si NP slurry wastes following CMP processing of toxic III-V materials are unknown. Our hypothesis is that the physicochemical characteristics and cellular response of post-CMP or spent NP waste will be very different in their state of aggregation, dispersibility and charge, compared to pre-CMP or pristine NP slurries. In this work, we will report on (a) differences in the measured physicochemical properties of pristine NP slurries and spent NP waste after polishing of GaAs substrates, (b) differences in viability, cytotoxicity, uptake and oxidative stress of pristine CMP and spent slurries after exposure to A549 and RAW 264.7 cells and finally, (c) correlation of transformed physiochemical properties from CMP with their biological studies. This work was supported by funding (Task ID 425.051) from Semiconductor Research Corporation and ERC for Environmentally Benign Semiconductor Manufacturing at UofA.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Nanomaterial release, Physicochemical characterization

50. Intestinal Barrier Crossing Of SiO_2 And TiO_2 Nanoparticles: Set Up Of Experimental Conditions According To Nanoreg Protocols

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Crossing of biological barriers is a crucial aspect for nanomaterials (NMs) distribution within the organism. In NANoREG project capability of NMs to cross different in vitro models of epithelial barriers were investigated. Results will be used in the development of both decision tree and regulatory framework/toolbox, two of the main outputs of this project. We utilized differentiated Caco-2 cells as model of intestinal barrier. Crossing experiments were performed according to the common protocol developed in the project, taking into account NM peculiar characteristics. Special attention was given to preparation and characterization of NM batch dispersions and NM dispersions in culture media at the beginning and at the end of treatment. Dynamic Light Scattering (DLS) was utilized to characterize NM dispersions. DLS data of Z-Average and Polydispersity Index (PDI) showed a good reproducibility of batch dispersions of NMs in MilliQ-BSA (0.05%). NM dispersions in culture medium showed PDI and Z-Average values different from the batch dispersions, depending on exposure time and concentration of NMs. To verify if chemical composition might affect absorption profile, experiments were ran with two silica (NM200, NM203) and two titania (NM100, NM101) nanoparticles. None of selected NMs is toxic on Caco-2 cells (1-100 µg/ml) and none is able to alter intestinal barrier integrity. NMs crossing through Caco-2 barriers was evaluated by Scanning Electron Microscopy (SEM-EDX) technique, but NMs observation in cell medium is not easy due to the presence of high amount of organic background. Funding provided by FP7 NANoREG project, Grant n. 310584.

Keywords: Physicochemical characterization

51. Repeated Oral Administration Of Low Doses Of Silver In Mice: Effects On Central Nervous System

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Recently, in vivo studies underlined the potential neurotoxicity of silver nanoparticles (AgNPs) by inducing damage at the blood–brain barrier (BBB) and neurons. The aim of this study was to investigate the biodistribution and adverse effects on the CNS of low doses (0.25; 1 mg/kg bw) of 10 nm citrate-coated AgNPs and silver ions (silver acetate, AgAc) after repeated oral administration (28 days by oral gavage). ice were sacrificed at the end of treatment (28d) and after an equivalent recovery period (56d). At necropsy tissues were collected for histology and silver quantification by ICP-QQQ-MS analysis. Astrocytes (GFAP) and microglial cells (Iba1) were investigated by immunohistochemistry and transmission electron microscopy (TEM) was applied to assess BBB damage in the hippocampus. At 28d, the highest silver concentration was found in the brain, followed by liver and spleen. Tissue levels were slightly higher after exposure to AgAc than AgNPs, and dose-dependent. After the recovery period silver was still detected in the brain, at lower levels. An increase of GFAP and Iba1 immunoreactivity was detected in treated mice after 28d. TEM analysis demonstrated splitting of basement membrane of the capillaries, and swelling of astrocytic perivascular end-feet in treated groups. In conclusion our study highlighted accumulation and retention of silver in the brain after oral administration at low exposure levels. Immunohistochemical and ultrastructural investigations revealed morphological alterations involving the BBB and microglial cells indicating potential neurotoxic effects. Research supported by Fondazione Cariplo under the 2011 call "Nanoparticles, nanotechnologies and ultrafine particles".

Keywords: Biodistribution, In vivo toxicology, Metal/metal oxide nanomaterials, Systems biology/toxicology

52. Development and Use of an In Vitro Liver 3D Model toAssess Iron Oxide Nanoparticle Genotoxicity

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The increasing development and use of novel nanomaterials across various fields has led to the need for their risk evaluation to assess safety and provide insights into their range of toxicity mechanisms. An important area of focus is the sites of nanomaterial accumulation in the human body, one of which is the liver. In this study, a hepatic 3D in vitro model was developed consisting of HepG2 spheroids grown in a hanging drop. The growth and metabolic characteristics of the liver spheroids were established by evaluating their surface area, cell viability and metabolite secretion (albumin, urea and aspartate transaminase). Magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) nanoparticle genotoxicity was then assessed in the liver spheroids using the cytokinesis block micronucleus assay following a 24-hour exposure period. Optimal growth and metabolic characteristics were observed after 4-days of spheroid growth; this was therefore the time-point selected for initiating exposure to the test nanoparticles. The maghemite nanoparticles showed significant ($P<0.05$) increases in micronucleus formation from the 1 µg/mL dose, in the absence of cytotoxicity. The distribution of the maghemite nanoparticles was further investigated using scanning electron microscopy and synchrotron X-ray fluorescence. It was observed that the maghemite nanoparticles accumulated mostly at the periphery of the spheroids tissues. In comparison to 2D monolayers, 3D spheroid tissues provide an improved and more realistic test environment to conduct *in vitro* toxicity studies. This study has been collaboratively funded by Swansea University, UK, and Université Grenoble Alpes, France.

Keywords: Genotoxicity, In vitro toxicology, Metal/metal oxide nanomaterials, synchrotron radiation

53. Toxicity of Cadmium Oxide Nanoparticles in Human TK6 and HepG2 Cells Evaluated Using In Vitro Cytotoxicity Assays

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Cadmium oxide nanoparticles (CdO NPs) are among the most industrially used metal oxide nanoparticles. They have been widely used for medical diagnostic imaging, therapeutics and the manufacture of quantum dots. Thus, toxicity evaluation of CdO NPs for risk assessment is urgently required. In this study, we treated human TK6 and HepG2 cells with 10 nm CdO NPs and measured their cytotoxicity using several different in vitro assays including the MTS assay, ATP content detection assay, lactate dehydrogenase (LDH) assay and glutathione (GSH) luciferase assay. The endotoxin content was measured using the kinetic chromogenic Limulus Amebocyte Lysate (LAL) assay before the toxicity assays. The results showed that the endotoxin level within CdO NPs was below the limit of detection. CdO NPs induced concentration-dependent cytotoxicity in TK6 and HepG2 cells in the MTS, ATP and LDH assays. Exposure of CdO NPs to the cells also significantly decreased the reduced glutathione contents, suggesting that CdO NPs insult cells via increasing cellular oxidative stress. These results suggest that CdO NPs are toxic to human cells, leading to decreased cell viability and cell death. The mechanism underlying these toxicities of CdO NPs is possibly via producing oxidative stress in the exposed cells.

Keywords: Environmental nanotoxicology, Cadmium oxide nanoparticles, Environmental nanotoxicology, In vitro toxicology, Human cells

54. In Vivo and In Vitro Immunotoxicity of SiO_2 Nanoparticles

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An important concern regarding the safety of nanomaterials (NMs) is their interaction with the immune system which may affect NMs biodistribution, thus contributing to different biological responses. Conversely, NMs could affect viability and functions of immune cell populations, thus exerting undesirable side effects. So far, there is no specific harmonized procedure or regulatory guidance document currently available. As well, there is no simple test which can fully predict a NM's immunotoxicity. Therefore, in the framework of FP7 NANoREG project, NM203 SiO2 nanoparticle immunotoxicity has been studied by both in-vivo and in-vitro approaches. The following endpoints have been evaluated after a 90-day oral toxicity study in male and female rats (OECD Guideline 408) treated with 0, 2, 5, 10, 20, 50 mg/kg/day: immune organ weight (spleen, MLN); PHA-induced lymphocyte proliferation (spleen, MLN); lymphoid population analysis by FACS (spleen, MLN); LPS-induced NO and cytokine production (peritoneal macrophages); blood count; serum antibodies and inflammatory cytokines. Given its relevance for oral exposure, NM203 impact on intestinal barrier integrity was also evaluated using Caco-2 in-vitro barrier model. Immunotoxicity in-vitro studies included inflammatory and functional endpoints on the murine macrophage cell line RAW264.7: apoptosis/necrosis, cytokine secretion, NO production. Whereas in-vitro studies did not highlight significant toxicity on both Caco-2 barrier and macrophage cell line, the in-vivo results pointed to impaired lymphocyte response to mitogen associated with reduced numbers of circulating white blood cells, and enhanced inflammatory response by peritoneal macrophages; gender effects have been observed. This study has been partially supported by NANoREG project contract no.310584.

Keywords: In vitro toxicology, In vivo toxicology, Immunotoxicity

55. Multi-walled Carbon Nanotubes Trigger and Amplify Th2-type Immune Responses in Mouse Lungs

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Carbon nanotube (CNT)-induced lung fibrosing lesions are delineated by an acute inflammation followed by chronic interstitial fibrosis, for which the molecular mechanism is largely unknown. We performed a genome-wide microarray analysis of lungs exposed to single dose multi-walled CNT (MWCNT) by pharyngeal aspiration for 7 days and compared to vehicle and carbon black controls, to identify differentially expressed genes and mechanisms that potentially function in the development of CNT-induced lung fibrosis. Data analyses revealed T helper 2 (Th2)-driven innate immune responses were preferentially enriched. We demonstrated that MWCNT induced the expression of Th2 cytokines interleukin (IL)-4 and IL-13, and a panel of signature downstream target genes including Il4i1, Chia, and Ccl11/Eotaxin, at the protein level in the lungs. Induction of Th2 cytokines occurred in CD4+ T lymphocytes, indicating the activation of Th2 cells. Activation of Th2-type responses is featured by the activation of IL-4Ra/STAT6 signaling pathway in CD4+ T cells. Indeed, MWCNT significantly induced this pathway via phosphorylation of STAT6 and the up-regulation of GATA-3, which serves as a transcription factor and mediates the transactivation of Th2 target genes, in CD4+ T cells. Therefore, our study uncovered a novel molecular connection between acute inflammation and fibrosis development via Th2-driven immune responses following MWCNT exposure.

Keywords: Toxicological mechanisms, multi-walled carbon nanotube, inflammation, fibrosis, Th2-type immune response

56. E171 Food Additive and Titanium Dioxide Particle Toxicity on Gut Cell Models

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The food-additive E171 is a white pigment made up of TiO_2 particles and found in food products, such as sugar-coated gums and candies. It contains 30 to 40 % of nano-scale TiO_2 particles to which humans are thus exposed by ingestion. Nevertheless, the toxicity of these TiO_2 particles on gut cells remains poorly documented. To fill this gap of knowledge, our study focused on the impact of E171 on gut cells, in vitro. The study endpoints were the intestinal barrier function, i.e. ability to exclude toxicants from the intestinal lumen, mucus secretion and oxidative stress regulation. Particle toxicity was evaluated on two human cell models: differentiated Caco-2 enterocytes and a co-culture of Caco-2 cells with HT29-MTX mucus-secreting cells. These models were acutely or chronically exposed to fully characterized E171 or TiO_2-NPs. Their cytotoxicity (WST-1, trypan blue), genotoxicity (comet assay), and oxidative stress response (H2-DCF-DA) was monitored. Then, gene expression and content of proteins involved in oxidative stress and endoplasmic reticulum stress regulation, mucus production and ABC transporters were analyzed. E171 induced no overt cell mortality but significant oxidative stress and oxidative DNA damage. It also modulated the expression of genes, as well as the content of mucus proteins and ABC transporters. Taken together, these results suggest that high doses of TiO_2-NPs may alter the intestinal barrier function and thus be implicated in the development or aggravation of inflammatory bowel diseases. This work was funded by ADEME/CEA for the PhD grant, Labex Serenade and ANSES.

Keywords: Genotoxicity, In vitro toxicology, E171 food additive, TiO_2

57. Susceptibility of STAT1-deficient Mice to Pulmonary Fibrosis after Exposure to Tangled vs. Rigid Multi-walled Carbon Nanotubes

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Background: Multi-walled carbon nanotubes (MWCNT) pose a risk for pulmonary fibrosis due to their fiber-like shape, but physico-chemical features, like rigidity, could also confer fibrogenicity. The signal transducer and activator of transcription-1 (STAT1) is an anti-fibrogenic transcription factor that halts fibroblast growth. STAT1 deficient (STAT1-/-) mice are susceptible to pulmonary fibrosis. We hypothesized that STAT1-/- mice or primary STAT1-/- lung fibroblasts (MLF) exhibit a differential fibrogenic response to tangled (t-) vs. rigid (r-) MWCNT above that in wilt-type (STAT1+/+) mice or STAT1+/+ MLF. Methods: Primary MLF were isolated from STAT1+/+ or STAT1-/- mice and exposed to t- or rMWCNT (10ug/mL) for 24 hours. STAT1+/+ and STAT1-/- mice were exposed to t- or rMWCNT (4 mg/kg) via oropharyngeal aspiration and lung tissues were collected after one and 21 days. Both mRNA and protein samples were analyzed for fibrogenic mediators. Results: rMWCNT caused greater fibrosis and larger granulomas than tMWCNT. STAT1-/- mice treated with rMWCNT had higher collagen content and TGF-b1 cytokine levels after 21 days than STAT1+/+ after t- or rMWCNT exposure. STAT1-/- MLF had increased p53 expression compared to STAT1+/+ MLF; p53 protein levels were further increased by rMWCNT > tMWCNT. Conclusions: r- and tMWCNT induce different pulmonary fibrogenic responses that are exaggerated by STAT1 deficiency, emphasizing the importance of tube rigidity and genetic susceptibility. The mechanism of MWCNT-induced fibrosis in STAT1-/- appears to be through dysregulated TGF-b1 and p53 expression. Funding provided by NIH R01 ES020897.

Keywords: Carbon-based nanomaterials

58. Cytotoxicity, Genotoxicity and Mutagenicity Assessment of Titanium Dioxide Nanoparticles and Multi-walled Carbon Nanotubes

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We have studied the behavior of two representative engineered nanomaterials (NMs), titanium dioxide TiO_2 and multi-walled carbon nanotubes (MWCNT), which possess unique physico-chemical properties but also has raised concerns about possible environmental and health hazards. Potential health risks involving damage to DNA are among the most critical. Nanoparticles (NPs) in general are known to contribute to cellular toxicity and genotoxicity either directly or via the induction of oxidative stress. A range of in vitro assays were applied to study the impacts of TiO_2 and MWCNT NPs on various toxicity endpoints in different cell lines. We used the comet assay combined with formamidopyrimidine DNA glycosylase (Fpg) to measure DNA strand breaks and oxidized purines. The HPRT gene mutation assay was also used. We have demonstrated concentration- and time-dependent increases in toxicity, for both NMs. We also found that exposure to either TiO_2 or MWCNT caused intracellular production of reactive oxygen species. In addition, we observed a clear concentration-dependent increase in the frequency of HPRT-mutants after treatment of cells with MWCNT, whereas TiO_2 did not show a mutagenic effect. Our results indicate that a 24 h incubation alone is not sufficient to detect damaging effects of certain NMs on DNA (a shorter incubation time also being required); and the use of Fpg allows detection of oxidative damage to DNA. In conclusion, our data support a potential genotoxic/carcinogenic risk associated with MWCNT exposure. Supported by FP7 NANoREG (NMP4-LA-2013-310584), and by the Research Council of Norway, project NorNANoREG (239199/O70).

Keywords: Carbon-based nanomaterials, Emerging nanomaterials, Environmental nanotoxicology, Genotoxicity, In vitro toxicology

59. Role of Protein Corona and Complement Activation on the Induction of the Proinflammatory Response by IONP: An In Vitro and In Vivo Approach

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Protein corona (PC) is the main entity of initial cell interaction and can define the toxicological response to Fe_3O_4 nanoparticles (IONP). Protein interaction could activate the complement system, which in turn can yield to an inflammatory response and promote IONP-sequestration Polymer coating to IONP, polyethylenglycol (PEG) and polyvinylpyrrolidone (PVP), is an accepted strategy to prevent toxicity and avoid excessive protein binding. We evaluated the role of PC of three different IONP (bare, PVP or PEG coated) in the induction of cytotoxicity and proinflammatory response in vitro and in vivo. THP-1 macrophages were exposed to either IONP or IONP with a PC. All three IONP showed cytotoxic effects, which in the presence of PC were abolished. IONP-PEG induced ROS production, mitochondrial dysfunction and proinflammatory cytokines release. In contrast, PC presence on IONP-PEG promoted a decrease in ROS and prevented cytokine secretion. Also, the PC presence reduced cell uptake for IONP-bare, but had no influence on IONP-PVP or IONP-PEG. Further, BALB/c mice were exposed intravenously to IONP (5 mg/kg of b.w.). Results showed that IONP-PEG induced significant increment of complement activation markers (C5a and C5b-9). Moreover, IONP-PEG induced a systemic proinflammatory response observed as the increment of IL-1β, IL-6, TNF-α in plasma. In contrast, no significant changes were observed with IONP-b and IONP-PVP exposure. Our results suggest that the PC shield the cells against the toxic effects (ROS and mitochondria dysfunction) induced by IONP-PEG, and its proinflammatory effects arise from a complement recognition and the release of soluble mediators, namely anaphylatoxins.

Keywords: Biocorona, In vitro toxicology, In vivo toxicology, Toxicological mechanisms, Immunotoxicology, complement activation, iron oxide nanoparticles

60. Nanosensors for the Detection of Bacterial Pathogens

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Nanotechnology is creating new discoveries in areas such as medicine, automotive, energy, agriculture, remediation, consumer products and the entertainment industry. Central to the core of sustainable nanotechnology is the need to develop characterization parameters, metrological tools, novel instrumentation, and protocols that can provide information on the interactions of engineered nanomaterials with biological and environmental systems. Conventional methods for assessing these parameters focus on the size distribution and effects. They are however unsuitable for the detection and quantification in complex matrices. We propose that quartz crystal microbalance (QCM) would be faster and efficient in the detection of bacterial pathogens such as E.coli. Since QCM is very sensitive to mass changes. The strong affinity of mannose-derived ligands can be recognized by the fimbrial of the E.coli, which provides the basis for designing a nanomaterial-modified QCM biosensor. FimH adhesions present in type 1 pili of Escherchia coli can bind to the mannose-derived ligands. P-aminobenzoic acid, 4-aminophenyl disulfide, and 4-aminochlorobenzoic acid were chosen to aid the synthesis of the mannose-derived ligands by the modification of mannose via reductive amination. The synthesized ligands are characterized with the H1 NMR and mass spectrophotometer, thus confirming the presence of the desired product. The derived ligands are tested for E. coli detection under flow condition on the QCM apparatus.

Keywords: Environmental nanotoxicology, Human toxicology, Systems biology/toxicology

61. In Vitro Assessment of Secondary Mechanisms of Genotoxicity Induced by Superparamagnetic Iron Oxide Nanoparticles

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The main focus of in vitro nano(geno)toxicology studies has been mono-cellular cultures applied to evaluate primary genotoxic mechanisms. This eliminates the ability to explore secondary mechanism of genotoxicity, such as that resulting in chronic activation of immune cells. The present study assessed the ability of both Fe_2O_3 and Fe_3O_4 dextran coated superparamagnetic iron oxide nanoparticles (dSPION) (2-100 µg/ml) to promote secondary mechanisms of genotoxicity. The study was undertaken initially in mono-cultured 16HBE14o- bronchial cells and secondly in 3D co-culture models consisting of 16HBE14o- and THP-1 macrophages. Promotion of chromosomal damage in 16HBE14o- was quantified by the cytokinesis blocked micronucleus assay alongside cytotoxicity evaluation. Cellular uptake was investigated by electron microscopy. In monoculture treatments DNA damage was redox state dependant; only Fe_2O_3 was capable of promoting an increased micronucleus frequency, the lowest observed effect at 4 µg/ml rising in a dose dependant manner. In comparison co-culture treatments resulted in genotoxicity induced by both nanomaterials, the lowest observed effect at 10 µg/ml with a dose dependant frequency increase to 100 µg/ml for both materials. Macrophages within co-cultures demonstrated uptake of both nanomaterials, however only Fe_2O_3 provided visible evidence of location within 16HBE14o-. Macrophage dSPION internalisation consequently promoted secondary mechanisms of DNA damage within 16HBE14o- cells, hence the ability of Fe_3O_4 to promote genotoxicity in co-culture treatments. This study confirms the importance of considering secondary mechanisms of DNA damage when assessing nanomaterial genotoxicity in vitro.

Keywords: Genotoxicity, In vitro toxicology

62. Long-Term Effects of Acute and Chronic Exposures of Surface-Functionalized Gold Nanoparticles at Low Dose on Human Cells

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The toxicological impact of gold nanoparticles (AuNPs) mostly focuses on the acute response, usually at high doses. In vitro studies evaluating the AuNPs long-term exposure effects are not well-explored. Thus, despite the ever-widening range of knowledge into the effects of NPs on cells, there are still many unknowns in the big picture applying more realistic dosages and well-characterized NPs. Herein, we report (to our knowledge) the first study comparing in vitro long-term effects of low dose of AuNPs (0.1nM) with varying shapes and surface coatings under both “non-chronic” (acute cell exposure, followed by 20 weeks in a NP-free cell media) and chronic (exposure to AuNPs continuously over 20 weeks) ways. Human dermal fibroblast cells were used. By coating Au nanospheres and Au nanorods in two different functionalities each (citrate or poly(acrylic acid) (PAA) and PAA or PEG, respectively) and also having a common surface coating (PAA) between the two NP shapes, cell viability, proliferation, morphology, NPs uptake and expression levels of 84 genes by qPCR were evaluated. Chronic PAA rods and PAA spheres affected very little cell viability. No effect was observed on cell proliferation. In a long-term, cells exposed to non-chronic PAA rods and non-chronic and chronic PEG rods presented increased areas. PAA rods were the most endocytosed NPs. The long-term evaluation of non-chronic samples showed more gene changes than chronic. Overall, genes related to antioxidant and proteotoxic stress pathways were upregulated. Non-chronic PEG rods exposure presented the most different pattern of gene expression and the highest number changes.

Keywords: Exposure assessment, Human toxicology, In vitro toxicology, Metal/metal oxide nanomaterials, Gold Nanoparticles, surface chemistry, long-term study

63. Support Tools for the US-EU NanoEHS Scrimmage on Nanomaterials Spill Scenario

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The US-EU NanoEHS Communities of Research (CoRs) [1] are preparing a new scrimmage which is envisioned to involve a version of the nanoEHS scrimmage event piloted at the 2015 meeting, updated and expanded based on the feedback from across all CoRs. The aim is to exchange expert opinions across scientific disciplines and practices organized as six nanoEHS CoRs. During the exercise, a problem scenario will be presented (e.g. nanomaterial-based products spilled on city streets and near a waterway) which requires the CoRs' interactions and to report out their results to different audiences (e.g. authorities, companies, general public). The eNanoMapper project [2], via the Databases and Computational Modeling CoR, supports the scrimmage structure development by defining the computer tools and platform to be used for this exercise and facilitating the scientific knowledge exchange. eNanoMapper expertise in developing data management and analysis infrastructure, together with ontologies supporting the nanosafety assessment activities, is being applied to the support solution. Various computational and web tools [3] but also nanosafety assessment approaches [4] will be incorporated in order to support the interactions of COR teams participating in the exercise, facilitating the dialogue between the expert teams, preparing the briefings, and recording the main outcomes of the discussions to maximize the exploitation of the scrimmage results with dissemination output. References: 1. US-EU Communities of Research (http://us-eu.org/); 2. EU FP7-eNanoMapper, Grant agreement no: 604134 (www.enanomapper.net); 3. Powers et al., Science of the Total Environment, 470–471, 2014; 4. Stone et al., Particle and Fibre Toxicology, 2014, 11:9.

Keywords: Environmental nanotoxicology, Exposure assessment, Hazard ranking/characterization, Metal/metal oxide nanomaterials, Nanomaterial release, Regulatory decision making, Risk assessment, US-EU NanoEHS Communities of Research

64. eNanoMapper - A Database and Ontology Infrastructure for Nanomaterials Design and Safety Assessment

Lucian Farcal[1], Barry Hardy[1], Markus Hegi[1], Haralambos Sarimveis[2], Philip Doganis[2], Christoph Helma[3], Nina Jeliazkova[4], Bengt Fadeel[5], Gözde Kilic[5], Gareth Owen[6], Egon L. Willighagen[7], Friederike Ehrhart[7], Linda Rieswijk[7], Vesa Hongisto[8], Penny Nymark[8], Pekka Kohonen[8], Roland Grafström[8]. [1]Douglas Connect GmbH; [3]In Silico Toxicology GmbH, Switzerland; [2]National Technical University of Athens, Greece; [4]IdeaConsult Ltd, Bulgaria; [5]Karolinska Institutet, Sweden; [6]EMBL - European Bioinformatics Institute, Germany; [7]Maastricht University, The Netherlands; [8]Misvik Biology Oy, Finland.

eNanoMapper [1] is developing a data management and analysis infrastructure together with ontologies supporting the safety assessment activities of the European nanomaterials research and development community. The project addresses the requirements of safety assessment of nanomaterials by providing databases, analysis tools and ontologies for risk assessment [2] and linking them with existing resources in this area. Ontology for nanosafety research is being developed to provide annotation of nanostructures and relevant biological properties, experimental model systems, conditions, and protocols, complex search and reasoning capabilities, and the integration of data from existing nanotoxicology sources [3]. eNanoMapper addresses issues of public domain and data sharing, and the establishment of a standardized schema and infrastructure for nanomaterials safety assessment [4]. The project catalyzes collaboration, integrated analysis, and discoveries from data organised within a knowledge-based framework. eNanoMapper supports the discovery of nanomaterial properties, and the identification of nano-bio interactions from linked datasets, ontologies and external data sources. By interfacing with statistical and data mining tools, eNanoMapper aims to provide scientifically sound guidelines for experimental design as well as computational models for predicting nanotoxicity [5]. These computational models will help to design safe nanomaterials and improve the risk assessment of existing nanoparticles.

References: 1. FP7-eNanoMapper, Grant agreement no:604134 (www.enanomapper.net); 2. http://enanomapper.net/applications; 3. Hastings J. et al., Journal of Biomedical Semantics, 6:10, 2015; 4. Jeliazkova N. et al., Beilstein J. Nanotechnol., 6, 1609-1634, 2015; 5. Tsiliki G, et al., RRegrs: an R package for computer-aided model selection with multiple regression models, Journal of Cheminformatics, 7:46, 2015

Keywords: Alternative testing methods/strategies, Human toxicology, In silico modeling, In vitro toxicology, Metal/metal oxide nanomaterials, Methods, Physicochemical characterization, Regulatory decision making, Risk assessment, databases, ontologies, modeling

65. Fabrication of Silver Incorporated Microcrystalline Diamond Films for Bactericidal Applications

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Nosocomial infections are expensive and responsible for millions of deaths per year. To decrease this problem innovative microcrystalline diamond films with silver nanoparticles incorporated were successfully elaborated, characterized chemically and physically, and tested for antibacterial capacity. Recent studies demonstrated that pure silver films are more effective antibacterial agents compared to microcrystalline diamond films. The incorporation of silver nanoparticles to the microcrystalline diamond films yielded a significant improvement in its antibacterial properties. In order to perform the bacterial characterization of these Micro Crystalline Diamond – Silver (MCD-Ag) films, a rigorous protocol for bacterial culture was executed and the development of the bacterial populations was assessed through growth curves and absorbance measurements with an ultraviolet-visible spectrophotometer. Furthermore, the technique of bacterial transfer was used to conduct a temporal quantitative analysis of the MCD-Ag bacterial inhibition properties resulting in zero bacterial growth within 24 hours. The work is supported by NSF-IFN, DoD, and UPR.

Keywords: Carbon-based nanomaterials, Composite nanomaterials, Developmental nanotoxicology

66. The Effects of Orally Administered Nanoparticles on the Gut Microbiota Homeostasis and Colitis Induction in Mice

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With the growing application of engineered nanomaterials (ENMs) in agri-food related industry, the potential impact of ENM gastrointestinal tract (GIT) exposure to human health need to be concerned. Herein, we investigated the effects of three kinds of agri-food related ENMs (AgNPs, TiO_2NPs and SiO_2NPs) on gut microbiota homeostasis and the potential colitis induction in mice after orally administration. Our study found that for 7-consecutive day oral administration of TiO_2NPs and SiO_2NPs at the levels similar to the general public exposure, no obviously histological changes and inflammation were found in colon of mice. However, the similar dose of AgNP could induce full-blown colitis in mice. Importantly, the investigation of the impact of orally administrated NPs on mouse gut microbiota indicates that AgNP oral administration may perturb in the composition of gut microbiota, inducing the reduction of Firmicutes/Bacteroidetes ratio. No strong perturbation of the gut microbiota composition was found in TiO_2NP-administrated mice. Acknowledgment: This work was supported by the National Natural Science Foundation of China (U1432245, 11275214 and 11475195).

Keywords: In vivo toxicology, Metal/metal oxide nanomaterials, Risk assessment

67. In Vivo Genotoxicity of SiO_2 Nanoparticle after Sub-chronic Oral Exposure

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The toxicity of a synthetic amorphous silica nanomaterial used as food additive (E551) was carried out with NM-203, provided by the EC-JRC, through a 90-day oral study (OECD TG 408) including genotoxicity assessment in different organs. Male and female SD rats were exposed daily by gavage to 0, 2, 5, 10, 20, or 50 mg/kg b.w. SiO_2. DNA damage was assessed by comet assay in bone marrow, intestine, colon, kidney, liver, spleen, ovary of females, and in blood, liver, bone marrow, spleen, testis of males. Pig-a gene mutations and micronuclei were measured in erythrocytes of males. No differences among groups in body weight gain and food consumption were detected. At the end of treatment, Si tissue levels were increased in a dose-related manner in spleen, whereas a high inter-individual variability was detected for the small intestine, liver and brain (with generally higher levels at the highest dose). No DNA damage was reported in blood, liver and testis of males while an increase was observed without dose response in bone marrow and a slight but significant dose-related effect in spleen. In females, spotted, dose unrelated, increase of DNA damage was observed in intestine, kidney, liver, spleen and ovary. No gene and no chromosome mutations were detected in blood and colon.
Partially financed by EU FP7 project NANoREG, grant 310584.

Keywords: Biokinetics/toxicokinetics, Genotoxicity, In vivo toxicology

68. Uptake, Cytotoxicity and Effect on Cell Monolayer Integrity of Two Similar Sized Titanium Dioxide Nanoparticles on the Human Intestinal Caco2 Cells

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Titanium dioxide nanoparticles are included into a wide panel of consumer products including paints, cosmetics and food additives. Nevertheless the toxicological evaluation on human health of these nanoparticles is still incomplete and the guidelines for toxicity assessment used for chemicals may be inappropriate partly due to interferences. Moreover the role of the various physic-chemical properties in the toxic effects observed remains to be clarified. In this project, the effects of two rutile forms of TiO_2, one hydrophobic (NM103) and one hydrophilic (NM104) onto human Caco2 intestinal cells were studied. We investigated: The uptake after acute and repeated exposure using transmission electronic microscopy; The cytotoxicity in both non differentiated and differentiated cells using MTS assay; The cell monolayer integrity using Trans Epithelial Electrical Resistance (TEER) and Lucifer Yellow transfer.Our results showed that both TiO_2 were uptaken by Caco2 cells after acute and repeated exposure and that the nanoparticles remained inside the cells even after a recovery period. No cytotoxicity was noticed up to 100 µg/ml after 24 hours exposure for both TiO_2 irrespective of the differentiation stage. After repeated exposure, interference issues limited toxic effects investigation. Finally, the Caco2 barrier was not affected after 24 hours exposure.We concluded that NM103 and 104 did not obviously induce acute toxicity on Caco2 cells but that cellular pathways alterations cannot be excluded as already shown by several authors. Supported by EU Framework 7 Programme (project NanoReg n°310584) and French National Research Agency (ANR 13-IS10-0005-01).

Keywords: Biokinetics/toxicokinetics, In vitro toxicology, Metal/metal oxide nanomaterials

69. Poly (Alizarin red S) Modified Graphene Screen Printed Carbon Electrode Combined with Polymer Based Solid-Phase Microextraction for Determination of Nicotine

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The electrochemical determination of nicotine was investigated at the poly (alizarin red S)-graphene/screen printed carbon electrode (poly (ARS)-GR/SPCE). The peak value of nicotine at poly (ARS)-GR/SPCE was increased comparing with the unmodified GR/SPCE, suggesting that the disposable GR/SPCE was efficiently modified by poly (ARS) to fabricate the good working area. Characterization of the modified electrode was realized with electrochemical impedance spectroscopy and scanning electron microscopy. Under ideal conditions, differential pulse voltammetry of nicotine showed oxidation at + 600 mV (vs. Ag/AgCl) in phosphate buffer solution pH 7.0. The standard calibration curve was achieved in the nicotine (NIC) concentration range of 30–1000 μM and the detection limit was found to be 4.6 μM at a signal-to-noise ratio of 3. The poly (ARS) modified disposable GR/SPCE can be employed for the direct evaluation of NIC in real electronic cigarette juice samples. Finally, the method was performed to the electrochemical assay of NIC in real-world water samples with satisfactory results.

Keywords: Composite nanomaterials, Nicotine, voltammetry, graphene, screen printed electrode, e-liquid analysis, water analysis.

70. Systematization of Toxic Properties of Nano-sized Metal Oxides using Neural Network Method

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Neural network models for prediction of cytotoxicity of nano-particles of metal oxides (with size between 15 and 90 nm) to bacteria Escherichia coli was employed in the study using the following descriptors: χ-metal electronegativity by Pauling scale, number of metal atoms in oxide, number of oxide atoms in oxide and charge of metal cation in oxide. It was illustrated how toxicity of metal oxides correlated with their chemical properties dependent on position of metal in Periodic table and composition of metal oxides. The results can be used as preliminary assessment of hazardous of metal oxides.

Keywords: Hazard ranking/characterization, In silico modeling, Metal/metal oxide nanomaterials

71. Does a Coating Matter? Antioxidant Enzymes Activities in the Water Flea Daphnia magna Exposed to Modified Copper Oxide Engineered Nanomaterials

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Production of reactive oxygen species (ROS) has been described as a general pathway of toxicity induced by various metal based engineered nanomaterials (ENMs) and other chemicals leading to oxidative stress. Coating-related changes in three antioxidant enzymes' activities in the freshwater flea Daphnia magna were assessed, based on information obtained in acute and chronic toxicity tests. Experiments on biochemical responses following exposures to different CuO ENMs were focused on the activities of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-Transferase (GST) after 2, 6 and 24 hours of exposure Enzyme activity responses varied across the ENM panel. SOD, CAT and GST, which are considered the most important antioxidant enzyme systems in invertebrate species, showed different responses across the different ENMs' surface modifications and time of exposure. Obtained results for CuO-PEG ENMs suggest a different mode of action compared to the other ENMs. Based on the results obtained it is apparent that ROS play an important role in the toxicity pathway observed, and the pattern observed depends on the CuO ENMs surface modification and time of exposure.

Keywords: Environmental nanotoxicology, antioxidant enzymes, copper oxide ENMs, coating, Daphnia magna

72. Numerical Algorithms for Supporting Qualitative and Quantitative Read-across for Nanomaterials

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Due to their extraordinary properties, nanomaterials offer promising avenues for future innovation. However, the same properties might result in anonymous risks to human health and the environment. Therefore in the development of nanotechnology, attention should be parallel focused on the promise of new possibilities and opportunities as well as on responsibility of industries to guarantee the safety of their products. Without doubts the probable hazardous impact of new nanoparticles should be identified before their mass production and application in the daily life. The conventional (i.e. experimental) risk assessment approaches are often expensive, time-consuming and inadequate for enabling safe use for newly developed materials in the fast moving market of nanomaterials. Thus, development of computational methods, complimentary to the experiments is of high interest. The most promising approaches that can be applied for this purpose are: Quantitative-Structure-Activity-Relationships (QSARs) and read-across. The successful concept and application of Nano-QSAR has been already demonstrated. However, there are serious limitations related to developing Nano-QSARs. The most significant limitations derive from the limited number and low quality of the available experimental data. In the absence of relevant and reliable data to build an appropriately validated Nano-QSAR model one may apply read-across. Unfortunately, the existing methods for performing read-across are rather expert-based estimations and do not guarantee the reliability and repeatability of interpolation/extrapolation. Thus the main aim of this study is to present novel and suitable numerical algorithms for predicting various toxicological activities of empirically untested nanomaterials based on limited data sets in qualitative and quantitative manner.

Keywords: In silico modeling, Methods, Risk assessment

73. PEG-coated Silver Nanoparticle Surface Activity and Lipid Monolayer Interactions

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Interactions between engineered nanoparticles (NPs) and biological molecules are key to understanding nano EHS. NPs can partition into lipid membranes and change their physical properties including lipid organization and permeability. This study investigates the effects of polyethylene glycol (PEG)-coated silver NPs on lipid packing using dynamic surface-pressure measurements and hyperspectral microscopy. Surface pressure-area (π–A) isotherms of the dioleoylphosphocholine/dioleoylphosphoglycerol (DOPC/DOPG; 1:1 mole ratio) lipid monolayers were examined as a function of NP concentration. Isotherms were conducted in the absence of

the lipid monolayer to determine the NPs surface activity and to distinguish between the surface activity of the excess coating polymer coating from coated NPs themselves. Our results show that the PEG-coated NPs are surface-active and compete with the lipids for adsorption at the air/water interface. In the absence of lipids, the NPs increase the surface pressure up to 45 mN/m upon compression before the NPs surface layer collapsed.

In the presence of lipids, the NPs and lipids act cooperatively to increase the π. At high NPs concentrations,

corresponding to the NPs completely covering the interface, the π–A isotherms more closely resembled that for NPs than for lipids. NP surface activity is predominantly (~80%) due to the excess coating material rather than the NPs themselves. Low NP concentrations lead to an increase in π due to increase lipid packing and the inherent surface activity of the NPs, and high NP concentrations lead to monolayer collapse due to lipid desorption from the interface. This work is supported by the National Science Foundation.

Keywords: Environmental nanotoxicology, Methods, Physicochemical characterization, Membrane interactions

74. Cytotoxicity and Therapeutic Effect of Irinotecan Combined with Selenium Nanoparticles

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Although chemotherapeutic drugs are widely applied for clinic tumor treatment, severe toxicity restricts their therapeutic efficacy. In this study, we reported a new form of selenium, selenium nanoparticles (Nano Se) which have significant lower toxicity and acceptable bioavailability. We investigated Nano Se as chemotherapy preventive agent to protect against toxicities of anticancer drug irinotecan and synergistically enhance the anti-tumor treatment effect in vitro and in vivo. The underlying mechanisms were also investigated. The combination of Nano Se and irinotecan showed increased cytotoxic effect with HCT-8 tumor cells likely by p53 mediated apoptosis. Nano Se inhibited growth of HCT-8 tumor cells partially through caspases mediated apoptosis. In vivo experiment showed Nano Se at a dose of 4 mg/kg/ day significantly alleviated adverse effects induced by irinotecan (60 mg/kg) treatment. Nano Se alone treatment did not induce any toxic manifestations. The combination of Nano Se and irinotecan dramatically inhibited tumor growth and significantly induced apoptosis of tumor cells in HCT-8 cells xenografted tumor. Tumor inhibition rate was about 17.2%, 48.6% and 62.1% for Nano Se, irinotecan and the combination of Nano Se and irinotecan, respectively. The beneficial effects of Nano Se for tumor therapy were mainly ascribed to selectively regulating Nrf2-ARE (antioxidant responsive elements) pathway in tumor tissues and normal tissues. Our results suggest Nano Se is a promising selenium species with potential application in cancer treatment.

Keywords: Biomedical/therapeutic applications, Selenium nanoparticle, Toxicity; Antitumor drug, Apoptosis, rf2-ARE pathway

75. Structural Effect and Mechanism of Carbon Nanomaterials as Efficient Antioxidant and Radioprotective Agents

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Owing to delocalized π double bond system, carbon nanomaterials (CNMs) have exhibited excellent capabilities in scavenging free radicals and radioprotection. However, little is known about the correlation between the structure of CNMs with their antioxidant capabilities and protective effects against radiation-induced damage.

Here, we describe a systematic investigation on the antioxidant and radioprotective effects of several typical carbon nanomaterials including $C_{60}(OH)_{28}$, $C_{70}(OH)_{28}$ and graphene quantum dots (GQD). Electron spin resonance spectroscopy (ESR) experiments confirmed all three CNMs could efficiently scavenge a series of free radicals and these scavenging capacities were related to their structures. In vitro assays demonstrated CNMs markedly decreased ionizing radiation-induced DNA damage and apoptosis. Further, we found that CNMs exhibited the radioprotection by decreasing the generation of reactive oxygen species, inhibiting the disruption of mitochondrial membrane potential. These differential protection efficiencies may be attributed to the differential electrophilic capacities, which are consequences of differing nanoparticle structures. Our work shows the correlations between the structure of CNMs their antioxidant capabilities, and protective effects against radiation-induced damage, which will be useful in guiding the design and optimization of nanomaterials for radioprotection and antioxidant-related bio-applications.

Keywords: Carbon-based nanomaterials, In vitro toxicology, Toxicological mechanisms

76. Determination of Toxicological Properties of Ester-based Core-multishell Nanoparticles

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In recent years, nanoparticles have increasingly found practical applications in medicine. Core-multishell nanoparticles (CMS-NP) as carriers are of special interest for dermal drugs because of the increase in skin penetration [1]. The aim of this study was to determine the toxicological properties including cytotoxicity (by MTT), ROS induction (by H2DCFDA and FACS) and the genotoxic potential (Comet-Assay) of three ester-based CMS-NPs differing in the inner alkyl chain length (CMS (10-E-12-350), CMS (10-E-15-350), and CMS (10-E-18-350)) and their components based on core-multishell architecture [2] in human primary keratinocytes. The analysed CMS components were the hydrophilic polyglycerol core (hPG), the lipophilic alkyl inner shells C12, C15 and C18, the hydrophilic outer shell methoxy poly(ethylene glycol) (mPEG350) and the combined inner and outer shell components mPEG350-C12, mPEG350-C15 and mPEG350-C18. Indeed the CMS-NPs CMS (10-E-12-350) and CMS (10-E-18-350) displayed a cytotoxic effect in dependency of the alkyl chain length after 24 h incubation. The composite of inner and outer shell mPEG350-C15 and mPEG350-C18 showed an alkyl chain length dependent cytotoxicity. A significant genotoxic effect was observed only for the CMS (10-E-18-350) and the combined inner and outer shell mPEG350-C18. This finding correlated with a significant increase of ROS production caused by the CMS-NP CMS (10-E-18-350) and the component mPEG350-C18. In conclusion, our data indicate a causal role of the composite inner and outer shell mPEG350-C18 for cytotoxic and genotoxic effects. Although the analysed CMS-NPs are structurally comparable, slight extensions of the inner shell alkyl chain resulted in significant increase of cytotoxicity and genotoxicity.

Keywords: In vitro toxicology

77. In vivo Formation of Ferritin Nanoparticles: Understanding the Up-regulation of Iron and its Corresponding Oxidation States as an Antioxidant Pathway to Offset Invader Nanoparticle-Induced Inflammation

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High ferritin protein serum blood levels have long been associated with inflammation. We demonstrate for the first time the in vivo format ion and buildup of ferritin iron nanoparticles (FINs) in the immediate surrounding areas of engineered nanoparticles that had translocated into organs. FINs represent bio-mineralized iron nanoparticles that are 5-12 nm in size and trapped inside the cage of the iron storage protein. High resolution scanning transmission electron microscopy (STEM) and electron energy loss spectroscopy (EELS) were used to analyze FINs inside cells. A spatial and temporal association of copious FINs with various invader nanoparticles (inhaled or intravenously administered CeO_2, Al_2O_3, SiO_2 and carbon nanotubes) will be shown in lung, liver, spleen and brain regions. Surprisingly, different invader nanoparticles, even after being delivered to various organ tissues via different uptake routes share a common phenomenon, the buildup of FIN-rich halos which were identified using EELS analysis. This close proximity and spatial arrangement has not been described previously. Potential mechanisms that control the abundant in vivo formation of FINs may relate to an inflammatory response caused by invader nanoparticles, triggering an upregulation of iron that explains the FIN-rich halos observed in STEM images. Therefore, the Exposure-Dose-Response relationship of invader nanoparticles has to consider the formation of FINs which may be linked to regions of high oxidative stress. The formation of FINs involves a transition from reduced to oxidized iron, an electrochemical process that can be involved in neutralizing free radicals. We will present 2D and 3D images and videos of FINs.

Keywords: Developmental nanotoxicology, Emerging nanomaterials, In vivo toxicology, in vivo nanoparticle formation

78. Intracellular Accumulation of Gold Nanoparticles Leads to inhibition of Macropinocytosis to Reduce the Endoplasmic Reticulum Stress

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Understanding the toxicity of nanomaterials remained largely limited to short-term cell-death based assays in vitro and analyses of tissue-level accumulation and clearance patterns in animal models, which have produced very little information about how these materials effect complex cellular machinery at molecular level. Toxicity caused by the intracellular accumulation of nanomaterials during prolonged exposure is regarded important yet still continue to be an unexplored question. In this regard, here, we investigate intracellular accumulation of gold nanoparticles (AuNPs) for over a two-month period at a constant dose below acute toxicity level. We report, for the first time, a steep accumulation of 12-nm-size gold nanoparticles inside vascular endothelial cells followed by selective inhibition of macropinocytosis, the main route of uptake for such particles, causing reduced uptake of the AuNPs. Therefore, the cells can effectively deplete the amount of intracellular AuNPs via cell division, and thereby reducing the endoplasmic reticulum (ER) stress, which is associated with the initial nanoparticle accumulation. Interestingly, the clearance mechanism was found to be irreversible. Upon reaching the maximum achievable intracellular dose, a steady depletion is observed with local fluctuations while no cell death is recognized. Consequently, this work demonstrates long-term accumulation track of gold nanoparticles at non-lethal toxicity dose as well as how cells implement uptake pathways to cope with the elevated intracellular stress by effectively depleting the intracellular nanoparticle amount. The Scientific and Technological Research Council of Turkey (TUBITAK) Grant no:213M001 and European Cooperation in Science and Technology (COST) Action: Modelling Nanomaterial Toxicity (MODENA).

Keywords: Biocorona, In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms, cell-nanomaterial interactions, gold nanoparticles, nanoparticle accumulation

79. In Vitro Models as Physiologically Relevant Tools to Investigate Pulmonary and Intestinal Toxicity

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The aim of our studies is to establish physiologically relevant in vitro models. These two in vitro models reproduce the alveoli (3D-tetraculture system) and small intestine barriers (co-culture). These two models are more physiologically relevant, compared to the systems used so far, as there is a surfactant secretion in the tetraculture system at the air-liquid interface (ALI) and a mucus production in the intestinal coculture. The tetraculture model was exposed to different realistic amounts of diesel exhaust particulate matter (80 ng/cm^2, or 240 ng/cm^2) at the ALI for different time. The exposure is obtained by coupling the tetraculture to the VitroCell system, which allows the generation of native aerosols. A clear dose-dependent translocation of the transcription factor Nrf2, which regulates gene expression in response to oxidative stress, was observed after 4h of incubation in the endothelial cells without reduction of cell viability. The intestinal coculture model was used to evaluate effects of Ag 20 and 200 nm particles on the metabolic activity, oxidative stress and pro-inflammatory cytokine release. Ag was found to be homogenously distributed in the cell with aggregates observed in specific locations for Ag 20 with a 5-fold increase in IL8 release. The proteomic data revealed that both Ag particles, Ag 200 at a lesser extent, induced oxidative stress pathways and affected cytoskeleton, but regulated different sets of proteins compared to $AgNO_3$. Therefore, these two systems may become valuable tools for toxicology in a physiologically relevant way.

Keywords: In vitro toxicology

80. A Redox Proteomics Approach to Investigate the Mode of Action and to Support Grouping of Nanomaterials

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As the numbers of nanomaterials (NMs) are steadily increasing test methods for screening and prioritization are urgently needed. In parallel, understanding modes of action of NMs is required to establish NM grouping. Oxidative stress is associated with different adverse outcomes of NMs and has frequently been identified in in vitro and in vivo studies. Different assays have been developed for this purpose. Here we have investigated a representative panel of 24 NMs including functionalized amorphous silica (6), zirconium dioxide (4), silver (4), titanium dioxide (3), zinc oxide (2), multiwalled carbon nanotubes (3), barium sulfate and AlOOH. Surface reactivities of all were studied in a cell-free system by electron spin resonance (ESR). NRK-52E cells were treated with all NM, analyzed for viability (WST-1 assay) and intracellular ROS production (DCFDA assay). Carbonylated proteins were analyzed on 1D and 2D immunoblots and identified by matrix assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF/TOF). Here we propose a comprehensive testing strategy for assessing the oxidative stress potential of NMs, which combines acellular methods and fast in vitro screening approaches, as well as a more involved detailed redox proteomics approach. This allows for screening and prioritization in a first tier and, if required, also for unraveling mechanistic details down to compromised signaling pathways. Furthermore it appears highly useful within a NM grouping strategy.

Keywords: Systems biology/toxicology, Toxicological mechanisms

81. In Vivo Biomolecule Corona Around Blood-circulating, Clinically-used Liposomes

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The spontaneous coating of nanoparticles by proteins, once in contact with biological fluids, has been termed the 'protein corona' (PC) and it is considered to be a determinant factor for their pharmacological, toxicological and therapeutic profile. This dynamic process is currently being extensively evaluated by the incubation of nanoparticles with plasma proteins. However, the extrapolation of in vitro formed PC to predict the fate of nanoparticles in vivo remains largely untested, therefore the comparison of in vitro and in vivo formed PC is of great importance. Our aim was to study PC formation onto clinically tested liposomal formulations (bare, PEGylated and targeted). The formation of in vivo PC was determined after the recovery of the liposomes from the blood circulation of mice. In comparison, in vitro PC was formed by the incubation of liposomes in mouse plasma. In vivo and in vitro formed PCs were compared in terms of morphology, composition and cellular internalization [1]. Moreover, protein exposure time is thought to greatly influence the composition of PC. For this reason, we attempted to offer a time-resolved, in vivo PC characterization of PEGylated liposomal doxorubicin, identical to the clinical product Doxil. The drug-loaded vesicles were injected into CD1 mice and recovered from the blood circulation 10min, 1h and 3h post-injection. The PCs formed at these three different time points were qualitatively and quantitatively compared [2]. Funding provided by Marie Curie Network PathChooser 10.1039/C5NR09158F.

[1] M. Hadjidemetriou et al., ACS Nano 2015, 9, 8142.; [2] M. Hadjidemetriou,et al., Nanoscale 2016, in press,

Keywords: Biocorona, Biomedical/therapeutic applications, Physicochemical characterization, Systems biology/toxicology, in vivo, mass spectrometry, electron microscopy, cellular internalization, liposomes

82. Positively Charged Nanoparticles Lead to Artifacts in a C. elegans Toxicity Assay

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The unique properties of engineered nanoparticles (ENPs) may lead to unforeseen interactions in toxicity assays designed for soluble chemicals. Yet, there are a lack of test methods tailored to ENPs, which has hindered efforts to understand the consequences of ENP release. Here, we detail our efforts to adapt a C. elegans toxicity assay for ENPs, including a cause-and-effect analysis to identify sources of error. We found that shaking plates during the assay decreased growth by 36% and altering feed concentration greatly affected toxicity of a positive control. We also found that different media produced similar results in the toxicity assay, allowing for flexibility to suit ENPs. Additionally, we discovered that positively charged polystyrene nanoparticles (PSNPs) impact growth of C. elegans. However, those impacts were due to an interaction with E. coli that is used as feed in the assay and impacts on growth were more variable for PSNPs (52%) compared to the positive control (9%). We repeated this test using Au ENPs with coatings ranging from positive to negative and found similar results with our positively charged Au ENPs compared to PSNPs, however, no impact was found with neutrally or negatively charged ENPs. These artifacts led us to test these ENPs in toxicity assays that do not require E. coli and we found no toxicity associated with the positively charged ENPs. We conclude that either a different assay must be used to accommodate positively charged ENPs or adaptations to the current assay must be made to avoid the interaction.

Keywords: Environmental nanotoxicology, In vivo toxicology, Methods

83. Comparative Analysis and Modeling of Relationships between Inherent Nanoparticle Characteristics and Observed Toxicity to Embryonic Zebrafish

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Understanding the inherent features of nanomaterials impacting their toxicity is critical to the development nanotechnologies that pose minimal threats to humans and the environment over the life cycle of the nanomaterial. We compared the results of several different models built to describe the relationship between inherent nanomaterial characteristics and the resulting toxicity to embryonic zebrafish (Danio rerio) found on the open-source Nanomaterial-Biological Interactions (NBI) knowledgebase at Oregon State University. Five different models developed using various statistical clustering techniques and the ABMiner predictive models were compared to identify the nanomaterial features most frequently related to toxicity. Nanoparticle core composition was found to be a significant contributor to toxicity in only one analysis, particle size was found to be important in 3 approaches, and outermost surface chemistry was identified as the common driver of toxicity in all five modelling approaches. Thus, classification of nanomaterials by simple descriptors such as core composition may not be sufficient for predicting nanomaterial toxicity or managing nanomaterial risks. These results highlight the benefit of large open-source data repositories and the value of comparing multiple modeling approaches to advance understanding of nanomaterial-biological interactions. Funding for this work was provided by the U.S. National Institutes of Health, Oregon Nanoscience and Materials Institute and Oregon State University.

Keywords: Developmental nanotoxicology, Hazard ranking/characterization, High throughput screening

Regulatory decision making

84. Measuring Particle Surface Property Transformations with the NanoTweezer Surface

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Optofluidics, USA.

The transformation of the surface properties of nanoparticles are crucial in determining nanoparticle fate. Current technologies, including DLS and Zeta Potential, have been used to monitor surface transformations, with little success. We present the development of the NanoTweezer surface, an instrument that uses the latest in microfluidics and nanophotonics to analyze the surface properties of individual nanoparticles in solution. The technology is designed to measure weak and non-ionic interactions, helping researchers build a more complete picture. We will discuss the background of the technology and present case studies measuring properties that cannot be attained with traditional particle analysis instrumentation, including protein corona measurements and degree of PEGylation. COI statement: We are a for profit institution commercializing technologies that we licensed to Cornell University and continue to develop.

Keywords: Biocorona, Environmental nanotoxicology, Hazard ranking/characterization, In vitro toxicology

85. Subcutaneous Cerium Oxide Nanoparticle Delivery Affords Similar Protection as Intravenous Delivery in Murine Model of Multiple Sclerosis

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Delivery of nanoparticles, including cerium oxide nanoparticles (CeNPs), into biological systems triggers immediate formation of a protein corona that influences the biodistribution and physicochemical properties of the nanomaterial. Custom CeNPs, stabilized with citrate and EDTA, have potent antioxidant activity, evident by reduced reactive oxygen species levels in the brains of intravenously treated mice with experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. The correlative reduction in symptoms and disease severity illustrates the therapeutic potential of this CeNP formulation. Here, we examined the efficacy of the less invasive and more patient-friendly subcutaneous administration route of CeNPs. Mice induced with the chronic progressive MOG35-55 model of EAE were treated with vehicle control or CeNPs: 15 mg/kg intravenously once on day 7 post-induction OR 10 mg/kg subcutaneously once per week (beginning day 7). Mice treated with subcutaneous CeNPs performed best on tests of motor function, though some protection was also afforded by the intravenous dose, relative to controls. Further, both groups of CeNP-treated mice exhibited significantly lower clinical scores compared to controls. Both subcutaneous and intravenous CeNP delivery resulted in cerium deposition in the brain, though subcutaneous delivery resulted in relatively high levels in the liver and spleen, likely due to the cumulative effect of multiple subcutaneous doses. Thus, subcutaneous delivery of these custom CeNPs does not seem to affect their ability to reach the brain and reduce EAE disease severity, suggesting that exposure to proteins in the subcutaneous environment does not alter the biodistribution and functional properties of this CeNP formulation.

Keywords: Biodistribution, Biomedical/therapeutic applications, Cerium oxide nanoparticles

86. At Equitoxic Concentrations, CuO Nanoparticles Lead to Higher Copper Body Burden in Daphnia magna Compared to Soluble Cu-salt.

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Copper nanoparticles (NPs) are used in antifouling paints and thereby pose realistic threat to aquatic biota. Daphnia magna is among the most vulnerable freshwater organisms to copper. As the toxicity of Cu-compounds is mediated by Cu^{2+}, organic ligands in the water reduce their toxicity by complexing copper ions. CuO NPs do not undergo complete dissolution if discharged into natural waterbodies thus may remain there for extended periods of time and disturb the ecosystem, e.g. via entering the food-web. The aim of this study was to compare the acute toxicity (OECD 202) of CuO NPs (25 nm) and $CuSO_4$ and copper body burden of D. magna in artificial freshwater (AFW) and in natural waters. CuO NPs were characterized with DLS, AAS and Cu-sensing bacteria. D. magna 48h EC50 in AFW was 1 and 0.05 mg Cu/L for CuO NPs and $CuSO_4$, respectively. Although CuO NP toxicity in natural water was up to 30-fold lower than in AFW, Daphnia copper uptake profiles and body burdens were comparable. Total X-ray fluorescence spectroscopy showed significantly higher copper accumulation for CuO NP-exposed compared to $CuSO_4$-exposed daphnids at equitoxic nominal exposure concentrations. 24h depuration did not lower copper body burdens to the level of unexposed controls. Our findings highlight the importance of paying more attention to the potential long-term effects since even significantly lower acute toxicity of metal NP may not equal proportionally lower hazard to the ecosystem. This work was supported by Estonian ETF9347 and IUT23-5 grants of the Estonian Ministry of Education and Research.

Keywords: Environmental nanotoxicology, Exposure characterization, Metal/metal oxide nanomaterials, Bioaccumulation, TXRF, Picofox, natural freshwater

87. A Comparison Study of the Mechanism of Nanotoxicity of Silica Nanoparticles in Human Primary Cells and Cancer Cells

Min Beom Heo, Minjeong Kwak, Nam Woong Song. Korea Research Institute of Standards and Science, South Korea.

These days, nanotechnology have become an indispensable techniques which are useful and effective for many kind of categories. The various nanoparticles are widely used in cosmetic, sunscreens, food packaging and medicine, etc. However, they can result in human health and safety risks because of their nanotoxicity. The cytotoxicity test of nanoparticles is positively necessary to recognize how dangerous they are. In this study, we confirmed the nanotoxicity of silica nanoparticles in human lung and liver cells. Also, cancer cell line that is being most widely used to test the cellular cytotoxicity and human-originated human primary cell line were analyzed to demonstrate the differences in mechanism. The analysis of mechanisms for nanotoxicity were determined by using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, flow cytometric assays and confocal laser scanning microscopy (CLSM). The results demonstrate that human liver cells are more sensitive than lung cells to the toxicity of 20 nm silica nanoparticles. Moreover, MTS assay results indicated that there was no difference between lung cancer and primary cells, not liver cells. These results can be used as a reference to evaluate the cellular cytotoxicity of various nanoparticles.

Keywords: Human toxicology, In vitro toxicology, Risk assessment

88. Neutrophil Plays Suppressive Roles in Pregnancy Complication Induced by Silica Nnanoparticles

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As the use of nanomaterials increases, there is growing need for ensuring the safety of nanomaterials. Particularly, it is essential to examine the reproductive and developmental toxicities of nanomaterials. Previously, we showed that silica nanoparticles (nSP) could induce pregnancy complication in mice, but the detail mechanism of nSP-induced pregnancy complication is hardly understood. It is reported that neutrophil, the first line of defense against microbial infection, might relate to pregnancy complication. In this regard, we previously demonstrated that nSP induced elevation of the number of neutrophil in peripheral blood. Here, we attempted to investigate the contribution of nSP-induced neutrophilia to the pregnancy complication. Pregnant BALB/c mice were intraperitoneally injected with PBS or anti-Ly-6G antibodies, which deplete neutrophils specifically, at gestational day 15. After 24 h, they were intravenously injected with nSP or saline. Depletion of neutrophil showed that exacerbation of the pregnancy complications such as decrease of maternal body weight or depression of fetus number in uterus in nSP-treated mice. Moreover, pathological histology analysis revealed that neutrophil depletion increased placental cellular damage. Thus, these results suggest that neutrophil could play suppressive roles in pregnancy complication, especially breakdown of maintenance of pregnancy induced by nSP. Our results provide important information about the mechanism of nSP-induced pregnancy complication.

Keywords: Composite nanomaterials, In vivo toxicology, Toxicological mechanisms

89. Identification of Biomarkers for Nano-safety Assessment through Proteomic Analysis of Multi-Walled Carbon Nanotubes Functionalized by Atomic Layer Deposition Coating

Gina Hilton, Alexia Taylor, Erinn Dandley, Gregory Parsons, James Bonner, Michael Bereman. North Carolina State University, USA.

Multi-walled carbon nanotubes (MWCNT) are commonly surface-functionalized post-synthesis to enhance their novel properties for use in electronics and engineering. Since MWCNT are known to cause lung fibrosis in rodents, it is important to determine how functionalization affects pro-fibrotic potential in order to predict and prevent fibrosis. Preventative measures can be acquired through the identification and monitoring of groups of protein biomarkers. The goal of this work was to use proteomics to identify biomarkers of fibrosis unique to MWCNT atomic layer deposition (ALD) coating type. Mouse epithelial cell culture was established using E10 cells derived from normal type-II epithelial cells. Cells were exposed to control media, uncoated (U)-MWCNT and two ALD-coated MWCNTs: aluminum oxide (A), and zinc oxide (Z). Following a 24 hour exposure, cells were harvested, lysed, trypsin digested, and the peptides were isolated. Nanoflow LC-MS/MS was conducted using a Q-Exactive Plus, and further validation of proteins was achieved using a triple quadrupole mass spectrometer, Quantiva. Spectra were searched using Sequest implemented through Proteome Discoverer. Skyline-daily was used to evaluate significant proteins of interest by selected-reaction monitoring. The E10 exposure yielded several differentially expressed proteins across coating types that perturbed pathways by different mechanisms; including: disruption in oxidative phosphorylation, and complement activation. A Welch t-test found the following number of significant proteins for each exposure group compared to control (α=0.05): 103 Z-MWCNT (5 μg/mL), 210 A-MWCNT (100 μg/mL), and 138 U-MWCNT (100 μg/mL). In-vitro proteomic experimentation was able to elucidate differential pathway disruption as a function of MWCNT coating type.

Keywords: Systems biology/toxicology, Proteomics

90. Silver Nanoparticles Exhibit Coating and Dose-Dependent Neurotoxicity in Glutamatergic Neurons Derived from Human Embryonic Stem Cells

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Silver nanoparticles (AgNPs) are used extensively as anti-microbial agents in various products, but little is known about their potential neurotoxic effects. In this study, we used glutamatergic neurons derived from human embryonic stem cells as a cellular model to study 20 nm citrate-coated AgNPs (AgSCs) and polyvinlypyrrolidone-coated AgNPs (AgSPs) induced neurotoxicity. AgSCs significantly damaged neurite outgrowths; increased the production of reactive oxygen species and Ca^{2+} influxes; reduced expression of the neuronal proteins MAP2, PSD95, vGlut1 and NMDA receptor proteins at concentrations as low as 0.1 µg/ml. In contrast, AgSPs exhibited neurotoxicity only at a higher concentration (5µg/ml). Furthermore, AgSCs increased the phosphorylation of glycogen synthase kinase-3 a/β at Tyr216 and Tau at Ser396 and reduced the expression of Tau46, which are typically observed in Alzheimer's disease. Our results demonstrate that stem cells can provide an excellent platform for studying neurodegenerative disease associated with nanoparticle exposure and developing new therapeutic strategies.

Keywords: Alternative testing methods/strategies, Metal/metal oxide nanomaterials

91. SUN Decision Support System: Incorporating Lifecycle Thinking in Nanotechnology Risk Control and Sustainability Assessment

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The early identification of the risks from engineered nanomaterials over the lifecycle can enable risk management at an early stage of product development. Toward this end, the EU SUN project integrates tools for risk analysis and evaluation of environmental, economic and social impacts into a user-friendly decision support system, tentatively named SUNDS. SUNDS comprises of two tiers of varying analytical complexity and data requirements. The first tier of SUNDS is based on the LICARA NanoSCAN that provides a semi-quantitative evaluation of risk and benefits. SUNDS Tier 2 implements an integrated Risk Control (RC) and a socioeconomic Assessment (SEA) modules. The RC module comprises three risk sub-modules dealing with environmental and human health risk analysis, while the SEA involves also Life Cycle Impact Assessment, Economic Assessment, and Social Impact Assessment. Within both the RC and the SEA modules, most of the sub-module outputs are organized according to four lifecycle stages: Synthesis, Production, Use and End of Life. RC also suggests Technological Alternatives and Risk Management Measures to appropriately reduce the identified risks. The SEA module pinpoints hotspots for TBL impacts based on technical thresholds and user preference profiles. A comparison of the number of hotspots within the lifecycle stages provides information on the most affected stage(s) where risk management action should be taken.

Keywords: Regulatory decision making, Risk assessment, Sustainability assessment

92. Silver Nanoparticles, Its Mechanism of Bacteriocidal Effect, Impact To Environment and Biological Toxicity to Humans

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We propose that the photocatalytic ejection of electron from the AgNPs undergoes a cyclic chain reaction resulting in formation of highly potent •O2- and •OH which act as the strong baceriocidal agents. In addition, we performed in vitro Ag+ ion release experiment. The AgNPs (particle size 60-80 nm, 2 g/10mL) were suspended in 10mL of PBS and irradiated with UV (245 nm) lamp hanging 5 cm over the AgNPs suspension. The released Ag+ was found to increase time dependently to 0.68 mg/mL at 55h suggesting it can provoke severe pollution problem and raise substantially very high impact to environment, in particular, the water pollution. We suggest that the increased utilisation of nanoparticles may lead to increased environmental contamination and unintentional ingestion via water, food animals, or fish. We also predict that Ag+ ions could interfere with the lysine- and sulfhydryl enzymes. Accordingly, evaluation of AgNPs must take into consideration not only absorption and extraintestinal organ accumulation but also the potential for altered gut microbes and the effects of this perturbation on the host. Conclusively, although Ag NPs may have a lot of beneficial effect, nanoparticles display several unique physicochemical properties that can interfere with or pose challenges to classical toxicity assays, hence its risk are increasing in parallel in view of its impact on the biological systems. To develop a reliable and fast assay method is urgently in need. In this regard, we suggest a simple in vivo GSH-erythrocyte hemolysis assay method to screen the toxicity of AgNPs.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

93. Magnetic Melanin Nanoparticles as Activatable Theranostics for PET/MRI/PA Tri-modal Imaging Guided Photothermal Therapy

Peng Huang, Jing Lin. Shenzhen Univeristy, P.R. China.

Background: The separation of tumor diagnosis and treatment is one of major issues in the clinic. Herein, we report a radionuclide 64Cu-labeled magnetic melanin nanopaticles (64Cu-MMNs) for multimodal imaging guided photothermal therapy (PTT), UV and γ irradiation protection. Methods: MMNs were synthesized by the biomimetic synthesis method using bioploymer melanin as the biotemplate. The radionuclide 64Cu was labeled with MMNs by the high affinity of metal ions of melanin. PET, MRI, and photoacoustic imaging (PAI) were carried out on U87MG tumor-bearing mice. PTT was conducted both in vitro and in vivo. Results: MMNs were synthesized by biomimetic synthesis method. The size of MMNPs is ~15 nm. The r2 value of MMNs is 167.28 mM-1 s-1, which is much higher than spherical magnetic nanoparticles. MMNs were radiolabeled with 64Cu and purified to give radiochemical yield ~100%. 64Cu-MMNs were stable in PBS and mouse serum up to 24 h. In vivo PET imaging showed high tumor uptake of MMNs after intravenous injection (150 μCi, about 10 %ID/g, 24 h). MRI and PAI also showed high tumor accumulation of MMNs. Afterwards, upon laser irradiation (808 nm, 0.5 W/cm2, 5 min), 100% tumor elimination was achieved in MMNs administered group (10 mg/kg of MMNs). MMNs also exhibit efficient shielding against UV and γ irradiation. Conclusion: MMNs showed great clinical translation potential as versatile biomimetic theranostic agent with multi-modality imaging capability and potent PTT effect, and as a potential radioprotector to shield the normal organs of cancer patients who are undergoing high dose radiotherapy.

Keywords: Biomedical/therapeutic applications, Theranostics, melanin, iron oxide, biomimetic synthesis, PET, MRI, photoacoustic imaging, photothermal therapy, UV protection, γ protection

94. A Black Phosphorus Nanosheet-Directed Photothermally Enhanced Chemotherapy

Peng Huang, Sheng Wang. Shenzhen Univeristy, P.R. China.

Near-infrared (NIR) light triggered photothermally enhanced cellular internalization of nanomedicine shows good potential to improve antitumor effect. In this work, a black phosphorus (BP) nanosheet-based nanomedicine, which is composed by BP nanosheet, human serum albumin (HSA) and paclitaxel (PTX), is developed for enhanced intracellular drug delivery. In this system, BP nanosheet is a photothermal agent and PTX is an effective antitumor drug, while HSA can serve as an exfoliating agent/stabilizer of BP nanosheet and a carrier of PTX. The BP nanosheets exhibit great photothermal performance, excellent biodegradability/ biocompatibility and good photothermal stability. Furthermore, under the NIR light irradiation induced mild hyperthermia (~43 °C), the cellular uptake efficiency of BP-HSA-PTX is enhanced, resulting in improved antitumor effect. These results suggest that this BP nanosheet-based nanomedicine is promising nanoplatform for cancer treatment.

Keywords: Biomedical/therapeutic applications, black phosphorus, photothermal effect, drug delivery, enhanced cellular uptake

95. Time-dependent Toxicity of CdTe Quantum Dots on Liver and Kidneys in Mice: Histopathological Changes with Elevated Free Cadmium Ions and Hydroxyl Radicals

Peili Huang, Zhiwei Sun, Ji Wang, Man Yang, Jilong Wang, Peijun Meng, Jiayi Li. Capital Medical University, P.R. China

A complete understanding of the toxicological behaviors of quantum dots (QDs) in vivo is of great importance and is a prerequisite for their applications in humans. In contrast to the numerous cytotoxicity studies investigating QDs, a paucity of in vivo studies of QDs have been reported, and the issue remains controversial. Our study aimed to understand QD-mediated toxicity across different time points and to explore the roles of free cadmium ions (Cd^{2+}) and hydroxyl radicals (•OH) in tissue damage. Male ICR mice were administered a single intravenous dose (1.5 μmol/kg) of CdTe QDs, whose liver and kidney functions and morphology were subsequently examined at 1, 7, 14 and 28 d. Furthermore, •OH production in the tissues was quantified by trapping •OH with salicylic acid (SA) as 2, 3-dihydroxybenzoic acid (2, 3-DHBA) detected by HPLC-fluorescence method. We used the induction of tissue metallothionein levels and 2, 3-DHBA/SA ratios as markers for elevated Cd^{2+} from the degradation of QDs and •OH generation in the tissues, respectively. Our experimental results revealed that the QD-induced histopathological changes were time-dependent with elevated Cd^{2+} and •OH and can recover after a period of time. The Cd^{2+} and •OH exhibiting delayed effects in terms of histopathological abnormalities. The histological assessments performed at multiple time points might facilitate the evaluation of the biological safety of QDs. We gratefully acknowledge the financial support of the National Natural Science Foundation of China (81273131, 81573201, 31471658) and Beijing Natural Science Foundation Program and Scientific Research Key Program of Beijing Municipal Commission of Education (KZ201510025027).

Keywords: Toxicological mechanisms, quantum dot; cadmium ion; metallothionein, hydroxyl radical, toxicity

96. Decreased Uptake and Enhanced Mitochondrial Protection Underlie Reduced Toxicity of Nanoceria in Human Monocyte-Derived Macrophages

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Cerium dioxide nanoparticles (nanoceria), currently used as catalysts including additives to diesel fuel, also present potential as a novel therapeutic agent for disorders involving oxidative stress. However, little is known about effects of nanoceria on primary human cells involved in the innate immune response. Here, we evaluate nanoceria effects on monocyte derived macrophages (MDMs) from healthy human subjects. Peripheral blood monocytes were matured to MDMs and exposed to nanoceria suspensions (0, 5, 10, 20 µg/mL) for 24 or 48 hours. We evaluated particle uptake, ultrastructural changes, cytotoxicity and mitochondrial damage in MDMs. Role of intracellular concentration of nanoceria in toxicity was evaluated by 3D image analysis and compared to monocytes as nanoceria sensitive cell model. Nanoceria failed to induce cytotoxicity in MDMs at the tested doses. Nanoceria exposed MDMs show no mitochondrial damage and display significant accumulation of anti-apoptotic proteins (Mcl-1 and Bcl-2) during the maturation process. TEM and confocal analyses revealed efficient uptake of nanoceria by MDMs, however 3D image analyses revealed lower nanoceria accumulation per unit cell volume in MDMs compared to monocytes. Taken together, our results suggest that mitochondrial protection and reduced volume-corrected intracellular nanoparticle concentration account for the lower sensitivity of human MDMs to nanoceria. Funding provided by Intramural Research Program of the NIEHS, NSF EF-0830093 and EPA DBI-1266252.

Keywords: Cerium oxide nanoparticles, Human toxicology, In vitro toxicology, Metal/metal oxide, nanomaterials

97. TiO_2 Nanoparticles Disrupt Attachment, Cytoskeletal Networks and Migration of Human Osteoblasts-like Cells in a Size Dependent Manner

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Background: Human exposure to titanium dioxide nanoparticles (nano-TiO_2) is increasing. Ti-based orthopedic and dental implants can release NPs upon abrasion. Little is known about how NPs interact with cytoskeletal protein networks and the functional/homeostatic consequences that might follow at the implant-bone interface with regard to osteoblasts. Aim: To study the effects of size of anatase nano-TiO2 on attachment and migration of SaOs-2 human osteoblast-like cells. Methods: SaOs-2 cells were exposed to clinically relevant concentrations (0.05, 0.5, 5 mg/L) of 5 and 40 nm spherical nano-TiO_2 for 24h. Impedance-based sensing (xCELLigence, ACEA) and scratch wound assay were used to assess cell attachment and migration, respectively. Fluorescent microscopy was employed to quantitatively analyze phosphorylated focal adhesion kinase (p-FAKtyr-397) – a marker for focal adhesion contacts (FAs), actin stress fiber length and microtubule networks. Results: Larger agglomerates were observed in the 5 nm-exposed cells. Time-dependent reduction of cellular attachment was associated with both NP treatments. Cells were smaller than control after NP treatment but retained normal morphology when treated with 40 nm and were elongated when treated with 5 nm. P-FAKtyr-397 analysis showed reduced size and number of FAs after NP exposure and more elongated FAs were associated with 5 nm nano-TiO_2 compared to controls and 40 nm NPs. Migration was significantly impaired in the 5 nm-exposed cells compared to controls. NP treatment appeared to disrupt the morphology of the cytoskeletal networks. Conclusion: nano-TiO_2 treatment disrupts SaOs-2 cells' size, morphology and migration, with smaller NPs having a more pronounced effect.

Keywords: Human toxicology, In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms, Titanium dioxide nanoparticles, anatase

98. Surface Modifications Of Silica Nanoparticles Affect Their Uptake By The Cells And Subsequent Pulmonary Toxicity

Gaku Ichihara[1], Sandra Vranic[2], Sahoko Ichihara[3], Wenting Wu[4], Eri Watanabe[1], Arisa Hayashida[1], Yurika Osada[1], Toshihiko Sakurai[1], Sonja Boland[5], Lang Tran[6], Gaku Ichihara[1]. [1]Tokyo University of Science; [3]Mie University; [4]Nagoya University, Japan; [2]University of Manchester; [6]Institute of Occupational Medicine, UK; [5]University of Paris VII Diderot, France.

It is necessary to clarify the relationship between specific properties of nanomaterial (NM) and induced biological effect, as this could lead to the development of NM “safe by design”. Silica nanoparticles (SiO_2 NPs) are one of the most exploited NPs nowadays. Due to their presence in the cosmetic additives, drugs and even food, a chance of exposure to these NPs is increasing, raising the question of their potential cytotoxicity. In this study we examined how different surface modifications of SiO_2 NPs affect their biological interactions. SiO_2 NPs of 25 nm in diameter were surface-modified by attaching amino groups, carboxyl groups or hydroxyl groups rendering them either positively, negatively or neutrally charged, respectively. Male C57BL/6J mice, 8 weeks old were exposed to 2 mg/kg or 10 mg/kg SiO_2 NPs by pharyngeal aspiration. After 24 hours, the bronchoalveolar lavage fluid (BALF) was collected to determine the total and differential cell count, protein levels and cytokine secretion. Cells taking up SiO_2 NPs were observed using confocal microscope. Positively charged SiO_2 NPs induced the increase in the number of total cells, neutrophils, total proteins, TNF-a and MIP-2. On the other hand, the negatively charged SiO_2 NPs didn’t induce any change in the levels of these parameters. The hydroxyl-modified SiO_2 NPs increased the number of total cells, neutrophils, total protein, TNF-a and MIP-2 only after treatment with the high dose of NPs (10 mg/kg). We could also observe the cells taking up SiO_2 NPs by the confocal microscope. We concluded that induction of the inflammatory response in the murine lung was dependent on the surface modifications of NPs.

Keywords: In vivo toxicology

99. Adjuvant Effects of Multi-walled Carbon Nanotube Sensitization with House Dust Mite Allergen Lead to an Exaggerated Asthmatic Phenotype in Mice

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Multi-walled carbon nanotubes (MWCNTs) have numerous applications in emerging technologies and there is evidence that they can have harmful effects upon inhalation. Of particular susceptibility are individuals with asthma, a lung disease characterized by a TH2 immune response, mucus cell metaplasia and airway remodeling. We hypothesized that intranasal (IN) exposure to tangled (t) or rigid (r) MWCNTs would sensitize the immune system to cause an asthma-like phenotype upon subsequent challenge with MWCNTs. Male C57BL6 mice were dosed via IN aspiration on days 0, 2, 4, 14, 16 and 18 with vehicle, HDM allergen (25 μg), t or rMWCNTs (0.5 mg/kg), or a combination of HDM and each MWCNT; n=29. Necropsy was performed on days 21-22 and bronchoalveolar lavage fluid (BALF), serum and organs were collected. BALF from HDM/MWCNT treated mice showed elevated leukocyte influx, with HDM treatment causing eosinophilia which was enhanced by MWCNT co-exposure. Serum IgE was enhanced in the rMWCNT/HDM group, but not the tMWCNT/HDM group, over vehicle and HDM. BALF levels of TGF-β1, OPN, IL-13 and IL-1β were not altered, while CCL2 was increased by HDM/tMWCNT treatment. Lung histology showed little effect of MWCNT treatment alone, while HDM caused lymphocyte influx, airway wall thickening and enhanced mucus production; these inflammatory effects were enhanced by HDM/MWCNT co-exposure. In conclusion, sensitization of mice with t or rMWCNT caused a strong adjuvant-like effect with HDM to promote an allergic inflammatory phenotype suggesting that t or rMWCNT represent a health risk to individuals with allergic airway disease. Funding: NIEHS grant R01-ES020897.

Keywords: Carbon-based nanomaterials, In vivo toxicology, Susceptibility

100. Comparison of Uptake, Cytotoxicity and Genotoxicity of Two Similar Sized Titanium Dioxide Nanoparticles on the Human Intestinal Cells Caco2 and the Hepatic Cells HepaRG

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Use of nanoparticles (Nps) in consumer products is increasing but the numerous manufactured Nps cannot be all investigated for their toxic effects in vivo. In order to get some key factors influencing their toxicity, in vitro studies are necessary. In this study, we tested two rutile TiO_2 of similar size (NM 103, hydrophobic and NM 104, hydrophilic), during 24h, in a range of 9 to 256 µg/ml, on human intestinal Caco2 cells and hepatic HepaRG cells, mimicking the organs of contact and accumulation respectively. Uptake was investigated by Transmission Electronic Microscopy (TEM) and aman microscopy, showing a large uptake of both NPs in the two cell lines but only in the cytoplasm, as a free form or trapped in vesicles. Using High Content Analysis (HCA), no increase of apoptosis, DNA damage and NFkB translocation was detected. Similarly, no induction of the genotoxicity was observed in the alkaline comet assay and the micronucleus assay. In contrary ELISA assay showed a slight increase of IL-8 release at the lowest concentrations for both NPs. Therefore, NM103 and 104 TiO_2 did not induce major acute toxic effects on human intestinal and liver cells. It must be highlighted that TiO_2 interferences have to be taken into account to get reliable results. Further work will investigate if any effect is induced at the molecular level. This project was funded by l'Agence Nationale de la Recherche (ANR-13-IS10-0005-01).

Keywords: Genotoxicity, In vitro toxicology, Metal/metal oxide nanomaterials, titanium dioxyde nanoparticles

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101. Induction of Immunogenic Cell Death by Mitoxantrone-loaded Iron Oxide Nanoparticles for Tumor Therapy Employing Magnetic Drug Targeting

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Innovative strategies fighting cancer by inducing long-term anti-tumor responses from the immune system are urgently needed. Previously, chemotherapeutics from the anthracycline class have been shown to induce immunogenic cancer cell death. Problematically, however, in systemic chemotherapy the patient`s immune system is severely impaired due to the unspecific action of the cytotoxic drugs precluding effective responses. To accumulate the drug exclusively in the tumor region and to reduce systemic side effects in healthy cells and tissues, we developed an iron oxide nanoparticle-based system for the magnetically-targeted delivery of mitoxantrone to the tumor, which has proven its long-term therapeutic efficacy in tumor bearing rabbits previously. In this study we show in vitro that iron oxide nanoparticles loaded with the chemotherapeutic drug mitoxantrone are able to induce cell death with immunogenic features comparable to the free drug, whereas unloaded nanoparticles exhibit excellent biocompatibility. Life-cell microscopy proves that the drug-loaded particles are able to enter cells and infiltrate multicellular tumor spheroids. We conclude that the targeted induction of immunogenic cell death exclusively in the tumor region by chemotherapeutics loaded nanoparticles might be a promising possibility to selectively modulate the tumor microenvironment and to stimulate immune responses against the tumor in the presence of an intact immune system in future clinical application.

Keywords: Biomedical/therapeutic applications, Metal/metal oxide nanomaterials, Toxicological mechanisms

102. Effects on Kidney Physiology and Nephrotoxicity from Extraordinary Urinary Excretion of Graphene Oxide Sheets

Dhifaf Jasim, Stephanie Murphy, Leon Newman, Rachel Lennon, Kostas Kostarelos. University of Manchester, UK.

Graphene-based materials have attracted great interest recently due to their unique properties that have led to a potential for use in many fields including biomedicine [1]. In this study graphene oxide (GO) derivatives (the most hydrophilic and biologically relevant 2D material) were administered intravenously in C57BL/6 mice. Extensive urinary excretion was one of the unexpected pharmacological findings [2], that indicated a strong interaction with the kidney glomerular filtration barrier (GFB). Analysis of the kidney function and histopathology was carried out. Serum and urine analyses revealed no impairment of kidney function up to one month after injection of GO at doses up to 10 mg/kg. Histological examinations suggested no damage to glomerular and tubular regions. Ultrastructural analysis by transition electron microscopy showed the absence of any damage, with no change in podocyte slit, endothelial fenestra sizes or glomerular basement membrane width. The data indicated that GO sheets with sizes exceeding by several times the reported GFB cut-off (<40 nm) were excreted. The study provides better understanding of how emerging 2D nanomaterials interact with biological barriers and shows that GO has potential in the development of various biomedical applications, such as the diagnostics and drug delivery.

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Keywords: Carbon-based nanomaterials, Emerging nanomaterials

103. Brain Targeting Drug Delivery Characteristics of HupA-PLGA-NPs

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Huperzine A (Hup A) is a naturally occurring compound found in the firmoss Huperzia serrata. Hup A is a licensed anti-Alzheimer's disease (AD) drug in China and a nutracetical in the United States. Nanoparticles drug delivery systems can improve the bioavailability of Hup A. Here, we tested the brain targeting drug delivery characteristics of HupA-PLGA-NPs. In vitro blood-brain barrier (BBB) model has been developed. The 46.4 nm HupA-PLGA-NPs showed higher rate of crossing BBB than that of 208.5 nm HupA-PLGA-NPs. Qualitative observation of Cou-6-PLGA-NPs with mean diameter 45.9 nm and 202.8 nm and quantitative research of HupA-PLGA-NPs with mean diameter 46.4 nm and 208.5 nm showed that both 50 nm and 200 nm PLGA-NPs were uptake by bEnd.3 in time-dependent and concentration-dependent manners. The results also suggested that nanoparticles were absorbed by a synergistic action with multiple factors and mechanism. However, different diameters of nanoparticles showed different intake patterns. The nanoparticles with mean diameter about 50 nm were assimilated by bEnd.3 cells mainly through micropinocytosis and active transport depending on the energy, while the nanoparticles with mean diameter about 200 nm were taken into bEnd.3 cells through the clathrin-mediated endocytosis and caveolae. The bEnd.3 cells exhibited higher uptake index of HupA-PLGA-NPs with mean diameters about 200 nm than 50 nm. Besides, our research also found that HupA, HupA-PLGA-NPs of 46.4 nm and 208.5 nm showed no toxic effect to bEnd.3 cell at the concentration range of 5 ng/mL to 5 μg/mL.

Keywords: Biomedical/therapeutic applications, Huperzine A; PLGA-NPs, Blood-brain barrier

104. Interference of Gold Nanorod Core/silver Shell Nanostructures on Steroidgenesis in Primary Rat Granulosa Cells: Implications for Reproductive Toxicity of Silver Nanomaterials

Xiumei Jiang[1], Liming Wang[2], Yinglu Ji[3], Xin Tian[1], Xiaochun Wu[3], Chunying Chen[3], Jun-jie Yin[1]. [1]Center for Food Safety and Applied Nutrition, US Food and Drug Administration, USA; [2]Institute of High Energy Physics, Chinese Academy of Sciences; [3]National Center for Nanoscience and Technology, P.R. China.

Silver nanomaterials have been widely used in daily products and attracted great research interests. On the other hand, the potential hazard effects of these nanoformulations to human health have raised concerns. To date, the toxicity of silver nanomaterials to mammalian reproductive system is yet to be characterized. Therefore, we herein synthesized a gold nanorod core/silver shell nanostructure (Au@Ag NR) to study the cytotoxicity and steroidgenesis effects of nanosilver on primary rat granulosa cells. Our results suggest that Au@Ag NR were taken up into granulosa cells and translocated into lysosomes. Compared with gold nanorod (Au NR), Au@Ag NR induced a concentration-dependent decrease of granulosa cell viability. Although both Au NR and Au@Ag NR increased intracellular reactive oxygen species production and decreased mitochondrial membrane potential, only Au@Ag NR decreased the cellular Adenosine triphosphate (ATP) production. Furthermore, Au@Ag NR exposure significantly induce mitochondria-mediated cell apoptosis and necrosis. Both progesterone and estradiol secretion are increased after Au@Ag NR exposure in a time and concentration-dependent manner. In accordance, a time and concentration-dependent up regulation of steroidgenesis regulation proteins including FSHR, StAR, P450scc and p450arom were observed. In conclusion, our study suggests the silver nanomaterials exposure to rat granulosa cells would result in mitochondrial damage, cell apoptosis and disturb the progesterone and estradiol production.

Keywords: In vitro toxicology, apoptosis, steroidgenesis, reproductive toxicity

105. Size-dependent Gold Nanoparticle Uptake in Caenorhabditis elegans by Elemental Mass Spectrometry and Imaging Techniques

Monique Johnson, Antonio Montoro Bustos, Bryant Nelson, Shannon Hanna, R. Dave Holbrook, Christopher Sims, Lee L Yu, Karen Murphy, Michael Winchester. National Institute of Standards and Technology, USA.

Here we present results of size dependent uptake of AuNPs by C. elegans. Nematodes were exposed to 80 nm, 100 nm, and 150 nm AuNPs for 24 h at equal Au mass fraction concentrations. Common washing procedures were previously determined to be insufficient in removing excess suspended AuNPs after exposure, therefore, a sucrose density gradient centrifugation protocol was employed to separate the nematodes from AuNPs freely suspended in the exposure media. Image analysis of samples in all exposure conditions by scanning electron microscopy combined with energy dispersive X-ray spectroscopy (SEM/EDX) was performed to assess the efficacy of the C. elegans/AuNP separation. Quantification of total Au uptake in dry samples of non-exposed and Au exposed C. elegans was determined following acid digestions by conventional inductively coupled plasma mass spectrometry (ICP-MS) analyses. Alternatively, alkaline digestions of biological tissue were utilized for particle sizing in single particle ICP-MS analyses. Nanoparticulate body burdens were measured for each AuNP uptake condition. Finally, size distributions and particle number concentrations were determined for all AuNPs dispersed in water and AuNPs taken up by C. elegans. ICP-MS results suggest a size dependent AuNP uptake. Under these conditions, we found an increase in total Au uptake (related to Au concentration) for the 80 nm and 100 nm AuNPs, however there was reduced uptake for the 150 nm AuNP exposure. This reduction may be due to the decreases in total number of particles in solution with increased particle size.

Keywords: Biodistribution, Exposure assessment, Exposure characterization, Systems biology/toxicology

106. A Cross-species and Model Comparison of the Toxicity of Nanoparticles Used in the Pigment and Ink Industries: Informing the Development of Tiered Testing Strategy for Nanotoxicology

Helinor Johnston, David Brown, Birgit Gaiser, Vicki Stone, Teresa Fernandes. Heriot Watt University, UK.

A major user of nanoparticles (NPs) is the pigment and ink industry, where NPs are incorporated into numerous products (e.g. paints, food, plastics, printers, personal care products, and construction materials). In this study, we examined the toxicity of a panel of NPs, of varied physico-chemical properties, used in the pigment and ink industry (silver (Ag), iron oxide (FeO_3), titanium dioxide (TiO_2), zinc oxide (ZnO), cobalt aluminium oxide ($CoAl_2O_3$) and cadmium sellenide / zinc sulphide (CdSe/ZnS) quantum dots (QDs)) to mammalian cells (J774 macrophages, C3A hepatocytes and A549 alveolar epithelial cells) and aquatic environmental organisms (Raphidocelis subcapitata Daphnia magna, Lumbriculus variegatus). For mammalian cells, cytotoxicity was assessed 24 h post exposure, at concentrations ranging from 1-125ug/ml using the alamar blue and WST-1 assays. The aquatic toxicity of the NP panel was assessed according to OECD protocols (201, 202, 315), with some modifications to ensure their application to NPs, up to 96 h post exposure. A cross-species comparison revealed that Ag, QD and ZnO NPs were consistently more toxic than the other NPs tested. By studying effects across mammalian and ecotoxicological models we obtained a better understanding of the sensitivity of each model, and thus which models should be prioritised for selection in the future when assessing the mammalian and aquatic toxicity of NPs. The development of intelligent testing strategies for assessment of NP hazard is essential and data from this study can feed into the design of a tiered testing strategy for NPs.

Keywords: Alternative testing methods/strategies, Environmental nanotoxicology, In vitro toxicology, Risk assessment

107. Mechanism of Neutrophil Activation and Toxicity Elicited by Engineered Nanomaterials

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The neutrophil response to nanomaterials (NMs) is a key early step in the inflammatory process that influences the characteristics of inflammation such as intensity and duration. We evaluated the response of human HL60 neutrophil-like cells to NMs. It was hypothesised that NM physico-chemical characteristics would influence neutrophil cell responsiveness by altering intracellular Ca^{2+} concentration $[Ca^{2+}]i$ and reactive oxygen species production. Cells were exposed (1.95–125 µg/ml, 24 h) to silver (Ag), zinc oxide (ZnO), titanium dioxide (TiO_2), multi-walled carbon nanotubes (MWCNTs) or ultrafine carbon black (ufCB) and cytotoxicity assessed (alamar blue assay). NMs were categorised as being of relatively low (TiO_2, MWCNTs, ufCB) or high (Ag, ZnO) (cyto)toxicity. Sub-lethal impacts on cell function were investigated for TiO_2, Ag and ufCB. Ag was the most potent NM tested. Ag stimulated an increase in $[Ca^{2+}]i$ (Fura-2 loaded cells), and a prominent inward ion current (electrophysiology) and increased superoxide anion release. Ag stimulated cytokine production (MCP-1, IL-8) that was diminished by Ca^{2+} inhibitors or trolox, and exposed cells had an activated phenotype (light microscopy). ufCB stimulated an increase in $[Ca^{2+}]i$ but did not impact on the other parameters investigated. TiO_2 did not impact on cell function. NM toxicity was ranked Ag > ufCB > TiO_2. The battery of tests used provided insight into the mechanism of action of NM toxicity to guide future testing strategies. In addition the results obtained promote the use of alternative in vitro models, to better align nanotoxicology research with the 3Rs principles.

Keywords: Adverse outcomes pathway analysis, Alternative testing methods/strategies, In vitro toxicology, Toxicological mechanisms

108. Nanosilver-Induced Stress Responses in Unicellular Ciliate Tetrahymena thermophila

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Silver nanoparticles (AgNPs) are increasingly used in consumer products from where they could be released into the environment. Though numerous studies have demonstrated that Ag-ion release is the main toxicity mechanism of AgNPs, it is still debatable whether particle-specific effects also come into play. As the toxicity mechanisms may be organism-specific, we assessed the effects of protein-coated AgNPs (15 nm, solubility 2-3% at 100 mg/L), for the first time, on Tetrahymena thermophila – a protozoan that feeds by phagocytosis. AgNO3 and 26-nm polystyrene particles served as controls for Ag-ions and inert particles, respectively. Upon exposure to silver compounds for 2-24 h in deionised water T. thermophila showed high tolerance for AgNPs (24-h EC50=70-100 mg Ag/L). The EC50 for Ag-ions was 1.8-2.8 mg Ag/L. Upon exposure to low equitoxic concentrations of AgNPs and AgNO3 the expression of metallothionein genes in T. thermophila was time-dependently increased by the same fold, indicating Ag-ion driven toxicity. However, the expression levels of the stress-inducible and oxidative stress-related genes were higher in AgNP-exposed than in AgNO3-exposed ciliates. Also, compared to negative control AgNPs (but not Ag-ions) generated dose-dependently up to 7-fold increase in the levels of reactive oxygen species in the cell-free assays, revealing additional particle-driven toxic effects. The modulatory effects of the AgNPs and Ag-ions on oxidative stress-related enzymes activity in vitro will be reported.Support by Estonian projects IUT23-5, ETF9347 and COST Action BM1102 STSM Grant is acknowledged.

Keywords: Environmental nanotoxicology, In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

109. Protein Corona Analysis in Hepg2 Cells and Subcellular Fractions

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The protein corona constitutes the interface between a nanoparticle and the environment. It influences the bioavailability, toxicity and environmental fate of a nanoparticle. Superparamagnetic iron oxide nanoparticles (SPIONS) are being widely used for a plethora of biomedical applications. Therefore, the protein corona that formed around a carboxydextran-coated maghemite (Fe_2O_3- DEX) nanoparticle was analyzed. Separate analyses were carried out for the corona in HepG2 cells, in subcellular fractions of HepG2 cells, as well as in the cell culture supernatant. After incubation the protein corona complex was isolated via magnetization and the corona-forming proteins were identified by LC-ESI-QTOF MS/MS. Cellular uptake of nanoparticles was confirmed via fluorescence microscopy. After mass spectrometry analysis, 58 proteins were identified as the corona in HepG2 cells whereas 10 to 14 corona-forming proteins were identified in subcellular fractions, respectively. 65 corona proteins were identified in the cell culture supernatant. A large fraction of identified corona proteins is involved in cellular uptake mechanisms and shows nucleotide or metal binding affinities. In conclusion, the presented magnetization method is suited for the isolation and identification of the protein corona of SPIONS. Protein corona data indicate that uptake of carboxydextran-coated iron oxide nanoparticles occurs through a clathrin-mediated endocytose uptake mechanism.

Keywords: Biocorona, Metal/metal oxide nanomaterials, Uptake, HepG2, LC-ESI-QTOF MS/MS

110. Effect of Short Term Exposure of ZnO and TiO_2 Nanoparticles On Algae: Chlorella pyrenoidosa & Crustacean: Daphnia sp.

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Zinc oxide (ZnO) and Titanium dioxide (TiO_2) nanoparticles are some of the widely used nanoparticles (NPS) in commercial industry. Thus the chances for the particles to get run off to the aquatic environment and to there by affect the aquatic life are high. The present study evaluates the short term toxicity of ZnO NPs and TiO_2 NPs on Chlorella pyrenoidosa, which is a fresh water algae and on Daphnia sp. which is a common crustacean found in fresh water bodies. NPs were synthesized via chemical co precipitation technique and characterized UV-visible spectroscopy, X Ray Diffraction, XPS, Transmission Electron Microscope, Scanning Electron Microscope, Zeta sizer and Zeta potential analyzer. The dosage of the particles was from 0.001 mg/L to 100 mg/L. The study evaluated the chlorophyll content, lipid peroxidation, intracellular protein and extra cellular protein as a measure of toxicity for C. pyrenoidsa. The study found that the algae produced excessive amount of extracellular polysaacharides when it gets interacted with the NPs. The light microscopic evaluation also found the agglomeration of algal cells when interacted with NPS. The mortality of Daphnia sp was also studied both in the dark condition and also after the irradiation of sunlight. ZnO NPs was more toxic than TiO_2 NPs with Daphnia being more sensitive than Chlorella. The interacted organisms were observed under phase contrast microscope and the images were taken. The study was funded by Science & Engineering Research Board (SERB), Department of Science & Technology, Government of India (Registration No. SERB/LS- 587/2012).

Keywords: Commercialization, Environmental nanotoxicology, Exposure assessment, Exposure characterization, Risk assessment, Aquatic toxicity

111. Multilaboratory Evaluation of 15 Bioassays for (Eco)Toxicity Screening of 7 Engineered Nanomaterials: FP7 Project NANOVALID

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(Eco)toxicity of 7 well-characterized engineered nanomaterials (NMs) was evaluated by 15 bioassays in 4 laboratories. The highest tested nominal concentration of NMs was 100 mg/l. The bioassays yielded the following toxicity order: Ag>ZnO>CuO>TiO_2>MWCNTs>SiO_2=Au. Ag, ZnO and CuO proved toxic in the majority of the assays, assumingly due to dissolution. The latter was supported by parallel analysis of the toxicity of the respective soluble salts. The most sensitive tests/species were Daphnia magna (to Ag NMs), algae Raphidocelis subcapitata (ZnO and CuO) and murine fibroblasts BALB/3T3 (CuO): EC50=0.003 mg Ag/l; 0.14 mg Zn/l and 0.7 mg Cu/l, respectively. MWCNTs showed toxicity only to rat alveolar macrophages (EC50=15.3 mg/l) and TiO_2 to algae (EC50=6.8 mg Ti/l), assumingly due to high aspect ratio (MWCNTs) and entrapment of algae/agglomeration (TiO_2). Based on the decision-tree, for the screening and hazard prediction of NMs, we recommend a multitrophic suite of four assays: 48-h D. magna immobilization (OECD202), 72-h R. subcapitata growth inhibition (OECD201), 30-min Vibrio fischeri bioluminescence inhibition (ISO21338:2010) and 24-h BALB/3T3 neutral red uptake (OECD129) representing crustaceans, algae, bacteria and mammalian cells in vitro, respectively. Notably, these assays standardized for “regular”’ chemicals proved also efficient for shortlisting of hazardous NMs. Support by FP7 NanoValid and Estonian project IUT 23-5 is acknowledged.

Keywords: Alternative testing methods/strategies, Environmental nanotoxicology, Hazard, ranking/characterization, In vitro toxicology,

112. Inhalation of Multi-Walled Carbon Nanotubes Increases Heart Rate Variability Associated With an Alteration in Cardiac Function in Rats

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Objectives: Heart rate and cardiac function are regulated by the autonomic nervous system, and heart rate variability (HRV) is a marker of autonomic nervous system activity. The prognostic significance of HRV in cardiac disease has been reported in clinical and epidemiological studies. This study assessed the effect of inhalation of multi-walled carbon nanotubes (MWCNTs) on the autonomic nervous system and cardiac function. Methods: Male Sprague-Dawley rats were kept in the individual cages and exposed to MWCNTs for 5h at a concentration of 5 mg/m^3. The real-time ECGs was recorded by a telemetry system. HRV was assessed by analyzing beat-to-beat variations in RR intervals. Cardiac function was assessed in anesthetized rats at 1 day and 7 days post-exposure by a Millar catheter that was placed in the left ventricle and connected to a computerized system. Results: Compared to control group, MWCNTs exposure significantly increased the percentage changes in the root mean square of the successive differences (RMSSD) and high frequency (HF) during the exposure. Both the percentage changes of RMSSD and HF still remained high, but were not significant at 1 day-post exposure when compared with control group. Exposure to MWCNTs significantly decreased the percentage changes in stroke volume (SV), stroke work (SW), and cardiac output (CO) in response to dobutamine at 1 day-post exposure. Conclusions: Inhalation of MWCNTs significantly altered HRV and cardiac function. MWCNT exposure-induced changes in cardiac function were most likely due to the increased activity of parasympathetic nervous system.

Keywords: Carbon-based nanomaterials, In vivo toxicology, Systems biology/toxicology, Cardiovascular

113. The Sustainable Nanotechnology Organization (SNO)--5 Years of Professional Service

Barbara Karn, Wunmi Sadik. Sustainable Nano Organization, USA.

This poster presents an overview of the Sustainable Nanotechnology Organization (SNO) a relatively new professional science and engineering society focusing on both applications and implications of nanotechnology within systems leading to sustainability.Through a series of conferences, workshops and outreach activities, SNO has become a leading professional society in the area of using a new technology to aid sustainable development of new materials and consumer products and to ensure that the risk of these products is minimal both in their manufacture and in their exposure to humans and other organisms. SNO's programs include student travel awards to SNO conferences, a nanopitch contest, young faculty awards, a SNO award for exceptional contribution, topical workshops, special issues of journals, outreach to K-12 students, and soon will branch into student chapters. SNO communicates through its meetings, its newsletter (SNO Report), Facebook, workshops, and the Web. The poster presents a sample of this exciting organization's background and current activities.

Keywords: Emerging nanomaterials, Environmental nanotoxicology, Green nanomaterials, Life cycle analysis, Risk assessment, Sustainability

114. Single Cell Mechanics Provides an Effective Means to Probe Interactions between Cells and Engineered Silver Nanomaterials

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A critical step to understand and predict nanocytotoxicity is the knowledge of interactions between nanomaterials and cells. Cell-based assays have provided much information toward this goal. Given the complexity and variations of nanomaterial-cell interactions in vivo, single cell based methods are a pressing need to deepen our understanding, and bring us closer to predicting nanotoxicity. This work reports our multimodal approach to investigate nanomaterial-cell interactions at the single cell level. The engineered Ag nanomaterials include Ag nanoparticles 20 and 110 nm, and Ag nanowires, 20 μm long and 40 nm in diameter. The Ag nanoparticles are consortium-based samples from the NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR) Consortium. Exposure was by intratracheal instillation in rats at a single dose of 0.5 mg AgNPs/kg body weight, followed by a 24 hr recovery period. The consequences of nanoparticle exposure were investigated by harvesting alveolar macrophages (AM) from the lungs. Cellular mechanics measurements revealed diverse responses among AM cells, due to variations in AgNP uptake. Three major responses were evident: zero to low uptake that does not alter cellular mechanics, intracellular accumulation of AgNPs trigger cytoskeleton rearrangement resulting in the stiffening of mechanics, and damage of cytoskeleton that softens the mechanical profile. These effects were confirmed using confocal imaging of F-actin and measurements of reactive oxygen species production. In the case of nanowires, responses included frustrated phagocytosis, piercing of the cellular membrane, cytoskeletal reorganization, and deformation of cells. This work is supported by W.M. Keck Foundation, NIH and University of California, Davis.

Keywords: Alternative testing methods/strategies, Silver nanoparticles, Silver nanowires, Single cell mechanics

115. Comparative In Vitro Toxicity Study of Differently Sized and Charged Silver Nanoparticles on Yeast Saccharomyces cerevisiae BY4741 and Human Alveolar Epithelial Cells A549: The Role of Cell-Particle Interactions

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The widespread use of silver nanoparticles (AgNPs) in various antibacterial and antifungal products has led to the need for a wide range of toxicological studies to ensure the effective and safe use of nanosilver-enabled materials. The aim of this study was to evaluate the effect of size and charge of AgNPs on the cell-particle interactions and adverse outcomes to the yeast Saccharomyces cerevisiae (a fungal model) and human lung epithelial cells A549. Differently from the mammalian cells, yeast' cells have a rigid cell wall that might prevents NPs internalisation. However, similarly to mammalian's cells yeast can internalise NPs via active endocytosis. 10 nm and 80 nm sized, either citrate (negatively charged) or branch-PEI-coated (positively charged) particles were studied. Particle-cell interactions were assessed by confocal, scanning and transmission electron microscopy. We showed that 10 nm AgNPs were up to 40-times more toxic than 80 nm AgNPs, and bPEI-AgNPs were up to 100-times more toxic than cit-AgNPs to the yeast and A549 cells. Confocal microscopy confirmed the internalization of all the studied AgNPs by the both cell types. SEM and TEM visualization revealed the adsorption of bPEI-AgNPs (but not cit-AgNPs) onto the yeast' cell surface. Interestingly, both cit- and bPEI-coated AgNPs were adsorbed onto the surface of A549 cells, although the cit-AgNPs were not toxic to A549 cells (IC50~100 mg/mL). Results suggested that the toxicity of AgNPs depends on the size and charge but also on the type of the cells. This work was supported by Fondazione Cariplo (OverNanotox 2013-0987), ETF9001 and IUT 23-5.

Keywords: In vitro toxicology, Toxicological mechanisms, silver nanoparticles, cell-particle interaction, microscopy

116. In Vitro Assays Using Mussel Hemocytes as Valuable Tools to Detect Toxic Effects of Nanomaterials on Marine Organisms: The Case Study of Graphene Oxide (GO) and Reduced GO Nanoplatelets

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The use of graphene nanomaterials, such as graphene oxide (GO) and reduced GO (rGO), is in exponential increase. Thus, graphene-based wastes are expected to end up in the marine environment. Here we used in vitro assays with mussel (Mytilus galloprovincialis) hemocytes to assess the potential toxic effects of GO and rGO to marine organisms. Nanomaterials were characterized by AFM. Hemocytes were exposed to a wide range of concentrations of GO and rGO (with and without polyvinylpyrrolidone-PVP as stabilizing agent) to assess cytotoxicity. Then, cells were exposed to sublethal concentrations to assess intracellular localization through TEM and to evaluate their effects on plasma membrane (PM) integrity and ROS production. GO and rGO showed low and dose-dependent cytotoxicity, being rGO slightly more toxic than GO. PVP was not toxic to hemocytes but increased bioavailability of nanoplatelets. GO and rGO nanoplatelets were found in the cytosol and in endolysosomal vesicles. Nanoplatelets produced invaginations and perforations of the PM, causing a decrease in membrane integrity. Both GO and rGO increased ROS production at the highest sublethal concentration. In conclusion, GO and rGO cause damage to hemocytes PM and their toxicity is ROS-mediated. Chemical reduction of GO increases its bioreactivity, likely due to restoration of the unique electronic structure of the carbon atoms at the zigzag edges of platelets after reduction. In vitro assays with mussel hemocytes are sensitive tools to detect toxic effects of graphene-based nanomaterials. Funding provided by COST Action ES1205, Basque Government (consolidated group IT810-13, project IE14-393, postdoctoral fellowship) and UPV/EHU (UFI11/37).

Keywords: Adverse outcomes pathway analysis, Alternative testing methods/strategies, Carbon-based anomaterials, Environmental nanotoxicology, In vitro toxicology, Physicochemical characterization, Toxicological mechanisms, Graphene oxide (GO) and reduced GO, intracellular localization, membrane damage and ROS production, mussel hemocytes

117. Determination of IC_{50} of Two Different Nanomaterials in Male Germ Cell

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A number of nanomaterials have become available today and are being used in each and every domain of life. Their increased usage has drawn attention because of their associated side effects. Silver nanoparticles (AgNPs) and multiwalled carbon nanotubes (MWCNTs) have been used extensively in agriculture and livestock sector with respect to animal health, production, reproduction, prevention and treatment of diseases. Accordingly, it has become pertinent to evaluate their dosage limits in various model systems. Endorsing this hypothesis, the present work was designed towards determining IC_{50} for AgNPs and MWCNTs taking buffalo bull male germ cell, the spermatozoa as a cell model. Spermatozoa were treated with increasing doses of these nanomaterials (1, 10, 25, 50, 75,100, 200, 300 and 400 µg/mL) for different time intervals (30, 60 and 120 mins). Cell viability was assessed by MTT assay and eosin nigrosin staining. MTT reduction depends on the ability of metabolically active cells to reduce the tetrazolium salt to formazan - a colored product. Spectrophotometer readings obtained were converted to percentage viability values for each treatment group and were plotted against nanomaterial concentrations. Then by regression analysis equation, IC_{50} was obtained. IC_{50} for AgNPs was calculated to be 33 µg/ml,18 µg/ml and 3 µg/ml by MTT assay while 36 µg/ml, 21 µg/ml, and 8 µg/ml by eosin nigrosin method after 30 min, 1 hr and 2 hr of sperm nanomaterial incubation, respectively. On the other hand, IC_{50} for MWCNTs was 44.91 µg/ml, 21.98 µg/ml, and 6.8 µg/ml by MTT assay while 45.27 µg/ml, 22.19 µg/ml, and 7.1 µg/ml by eosin nigrosin method at 30 min, 1 hr and 2 hr of incubation respectively.Similar IC_{50} values obtained by two methods demonstrated the efficiency of each of them in determining half inhibitory concentration. Further, it was concluded that that AgNPs are comparatively more toxic than MWCNTs at least in the model system we checked in.

Keywords: Developmental nanotoxicology, germ cells, sperm, silver nanoparticle

118. Risk Assessment of Workers Exposed to Poorly Soluble Low Toxic Nanomaterials

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Even though safety of nanomaterials is still being discussed, it is required to conduct risk assessment/management with the latest available information. For Poorly Soluble Low Toxic particles (PSLTs), the US National Institute for Occupational Safety and Health estimated the Exposure Limit of TiO_2 based on the particle surface area metric converted from the mass of the lung burden. Also, efficient approaches for hazard assessment utilizing grouping of nanomaterials are under debate. On the other hand, as for exposure assessment, Organisation for Economic Co-operation and Development (OECD) proposed a tiered approach consisting of information gathering and exposure assessment, both basic and expert. We developed a method to calculate derived no effect levels (DNELs) using a lung burden particle surface area which was found to have a good correlation with Low Observed Adverse Effect Levels of TiO_2. And in assessing workplace exposure, we took a tiered approach recommended by OECD and conducted risk assessment by comparing DNELs with exposure levels to workers, taking protection factors for respirators into account. For estimation of exposure level, we used the mass concentration which we converted from the number concentration measured with an Optical Particle Counter, which we confirmed not to be an underestimation in the workplace. Our framework enables efficient risk assessment of PSLTs not only by assuming the worst cases in hazard and exposure assessment, citing the TiO_2 hazard data, but also by considering particle size, surface area and the operational condition in each workplace.

Keywords: Dosimetry, Exposure assessment, Occupational safety, Risk assessment

119. Inflammatory Effects Following Inhalation Exposure to Chemical Mechanical Planarization Slurries Containing Metal Oxide Nanoparticles

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The process of chemical mechanical planarization (CMP), used in semiconductor production, can result in the aerosolization of dispersant-stabilized nanoparticles (NP). Toxicological evaluation of metal oxide NP aerosols is essential to performing risk assessment for potential occupational exposures. We hypothesized that repeated inhalation exposure to aerosolized CMP slurries would lead to a dose-dependent pulmonary inflammatory response in male F-344 rats (200-300 g). Previous experiments tested a panel of different CMP slurries containing Al_2O_3, CeO_2, or SiO_2 NPs revealing that inhaled amorphous SiO_2 were the most inflammatory. Based on these results, the SiO2 NP slurry (31 nm) was selected for repeated whole body inhalation exposures (4 hrs/day, 5 days/week for 4 weeks) using ultrasonic nebulization at 3 different aerosol concentrations (0.2, 1 or 4.7 mg/m^3 as SiO_2; MMAD, 0.38-0.50 µm; GSD, 1.8-2.4). Retained lung dose was measured immediately following the last exposure (13.6 ± 3.3 – 196.3 ± 7.1 µg) and lung inflammatory responses were evaluated at 1, 7, and 27 days post exposure (PE). Lavage neutrophils stayed significantly elevated through 27 days PE for 1 mg/m3 and 4.7 mg/m^3 SiO_2 aerosol concentrations (4.2% ± 1.6% and 22.2% ± 1.3%, respectively). Thus, a no-observed-adverse-effect concentration was established at 0.2 mg/m^3 SiO_2. These results suggest that repeated exposures to SiO_2 NP-containing slurries can induce persistent lung inflammation, but that such outcomes may not be expected at typical lower workplace exposure concentrations. This research was funded by NIH P30 ES01247 and NY State Research Foundation subaward 13-15.

Keywords: In vivo toxicology, Metal/metal oxide nanomaterials, Occupational safety

120. Step-By-Step Altering of Physico Chemical Propoerties of Silica Nanoparticles and Their Impact on Cells

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Despite the widespread use of nanoparticles, our knowledge about possible risk factors is limited. Current toxicity assays do not specifically address nanoparticulate properties. We produced 12 different silica nanoparticles as a reference material. This library consists of two different sizes (100 nm and 250 nm), each of them with a low specific surface area and a high specific surface area. The particles additionally possess a negative, a neutral, or a positive surface charge. They were then tested regarding viability and oxidative stress at three different time points in a phagocytotic cell line (THP-1) and a non-phagocytotic cell line (HepG2). Interference of nanoparticles with the readout system, namely adsorptive, optical, and catalytical interference were tested. Furthermore, the hemolytic property of each particle type was assessed. The viability decrease due to silica nanoparticles was surface charge dependent, where a negative charge exhibits the strongest effect. A decreased viability was observed for particles with a high specific surface area compared to the ones with a low specific surface area. Additionally, the viability decrease and hemolysis was concentration dependent. In THP-1 cells, the viability loss was more pronounced for all particle types. In the given setup, no oxidative stress was detected. Negligible interference was observed. The most interesting candidate of this library was chosen for further in-depth studies.

Keywords: In vitro toxicology, Methods, Silica

121. TGF-B1 Mediated SWCNT Induced Lung Fibrosis Depends on the Upstream Osteopontin Stimulation

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Several studies have demonstrated that single-walled carbon nanotubes (SWCNT) exposure caused pulmonary fibrosis with a rapid inflammatory onset and subsequent granulomas formation through mechanisms involving epithelial-mesenchymal transition, myofibroblast ROS-dependent differentiation /recruitment accompanied by the release and interplay of various cytokines/chemotactic factors. The transforming growth factor-β (TGF-β) has been recognized as a central player in the robust pulmonary inflammatory response involved in the development of granulomas and interstitial fibrosis. However, the role of glycoprotein osteopontin (OPN) in TGF-β1 mediated fibrosis has not been fully explored. We used OPN-knockout (OPN-KO) and wild type (WT) C57BL/6 mice to investigate pulmonary fibrotic response upon exposure to SWCNT (40 μg/mouse). Reduced release of pro-inflammatory cytokines (MCP-1, TNF-α, IL-6), diminished pulmonary damage markers, and less pronounced neutrophil accumulation were found in broncho-alveolar lavage (BAL) of OPN-KO mice as compared to WT mice. Morphological examination revealed markedly decreased formation of granulomatous lesions along with diminished collagen deposition in the lungs of OPN-KO mice. While a significant increase in the level of TGF-β1 was found in BAL of WT mice, TGF-β1 readings in OPN-KO animals remained unaltered. In line with this, significantly reduced levels of TGF-β1 were detected when RAW 264.7 cells and MLE-15 cells exposed to SWCNT (24 hours, 6 μg/cm^2 to 48 μg/cm^2) were pre-treated with an OPN-blocking antibody. To the best of our knowledge, this is the first report to demonstrate that OPN may play a crucial role in TGF-β1 mediated SWCNT induced lung fibrosis. Supported by grants NORA 933051G and FP7-NANOSOLUTIONS no. 309329.

Keywords: Carbon-based nanomaterials, In vitro toxicology, In vivo toxicology, pulmonary fibrosis

122. Application of 3D A549 Cells on Evaluating the In Vitro Toxicity of Nanoparticles and High-throughput Screening of Nanotoxicity

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To assess the potential toxicity of manufactured or engineered nanoparticles, various cell model including cancer cell lines, primary cells or bio-engineered cell models have been applied. It has been reported that 3 dimensional (3D) cells are more functional and provide the biological relevant results comparing to traditional 2D cells. Traditional in vitro toxicity studies produce a false assessment of toxicity because nanoparticles have their own optical property which is interfered with light absorption or fluorescence. Here the new assay platform is applied to evaluate the in vitro toxicity of nanoparticles using 3D A549 cells on pillar insert. 3D cells based on pillar insert provide more in vivo mimicking state and to allow us to easily change cell growth media or expose 3D cells to detecting reagents by immersing the tip of the pillar insert in different reaction plates. This assay platform excludes the optical interference of nanoparticles and reactivity with the reagent of assay by reacting the exposed 3D cells into independent culture wells after nanoparticle exposure. Using this system, we evaluated the several kinds of nanoparticles including SiO_2, Ag, ZnO, and QDs. Here we provide the toxic effects of nanoparticles in 3D cells comparing to 2D cells and suggest the new assay platforms could be applied to the high-throughput screening of nanotoxicity.

Keywords: In vitro toxicology, Nanoparticles, In vitro toxicity, High-throughput screening, Insert pillar, Inverted culture, 3D cells

123. Impact of Metal Nanoparticles on the Increased Susceptibility of Welders to Bacterial Lung Infections

Jong Sung Kim, Adam Aitchison, Eileen Burns, Jason Leblanc. Dalhousie University, Canada.

Streptococcus pneumoniae (S.p.) infection is the most common cause of pneumonia and invasive pneumococcal disease (IPD), and a leading cause of death worldwide. Moreover, welders are at increased risk of developing and dying from S.p. infections; thought to be a result of chronic overexposure to metal nanoparticles (NPs) in welding fumes. These metal NPs carry many poorly understood risks that can negatively impact the health of welders. The primary goal of this study is to determine the biological mechanisms by which metal NP exposure increases the susceptibility of welders to infection by S.p. First, we will determine whether metal NPs function as a nutrient in promoting bacterial growth. We will expose S.p. serotypes (e.g., 4 and 8) to iron and copper NPs (5 nm) using a spark NP generation system for 1 h. Optical density (OD) will be measured every hour for 8 h, and the growth rate will be determined from a plot of the OD versus time. Second, we will determine whether metal NP exposure promotes adhesion of S.p. to human A549 lung epithelial cells, enhancing infection. Briefly, A549 cells will be exposed to iron and copper NPs (5 nm), followed by incubation with increasing doses of S.p. (101, 102, 103, or 104 CFU/mL). Viability of A549 cells and cytotoxicity will be measured. With successful completion of the work proposed here, I expect to provide valuable information on the underlying pathogenic mechanisms that will ultimately aid in disease prevention strategies.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Susceptibility, Toxicological mechanisms, Streptococcus pneumoniae, Welders, invasive pneumococcal disease

124. In Vitro Cardiopulmonary Toxicity of Airborne Copper-Based Nanoparticles

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Inhaled nanoparticles (NPs, <100 nm) may be important in cardiovascular effects because of their very high deposition efficiency in the pulmonary region, and their high propensity to penetrate the epithelium and reach interstitial sites and blood circulation. The respiratory and cardiovascular systems are intimately linked; thus NP exposure risks cardiopulmonary injury and requires a systems based approach of study. Little is known, however, about cardiopulmonary toxicity of pulmonary exposure to NPs due to the lack of an appropriate cell-NP exposure system. The long-term goal of this project is to better understand adverse health effects of NPs on respiratory and cardiovascular systems by providing more information on its causal factors and mechanisms. We used a spark discharge system capable of generating and delivering airborne metal NPs directly onto lung cells at an air-liquid interface. The generated NPs were characterized by using a SMPS, ICP-MS and electron microscopes. To better model *in vivo* repeated-low dose protocols we sequentially exposed lung cells to NPs *in vitro* (4 h exposure-2 h rest in an incubator-4 h exposure) and cell viability was determined by Alamar Blue assay. Our NP exposure system produced stable Cu NPs aerosols for 4 h (Cu, 2 x 10^6 particles/cm^3). Particle size distribution indicated the geometric mean diameter of the generated particles to be average 5 nm with a geometric standard deviation of 1.2. This research will aid in both understanding the impacts of NPs on human cells, and determining the mechanism responsible for cardiovascular disease following pulmonary exposure to NPs.

Keywords: Alternative testing methods/strategies, In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

125. Long Term Exposure to Cellulose Nanocrystals Enhance Morphological Transformation of Human Lung Epithelial Cells

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Cellulose nanofibers have gained much attention due to their biodegradable nature, expedient chemical and mechanical properties, economic value and renewability.However, before these materials can be considered for potential uses, investigation of their toxicities is required. This is especially important because cellulose nanocrystals (CNC) have a high aspect ratio and stiffness, features that are shared by asbestos and carbon nanotubes that are linked to high toxicity and potential to induce cancer. Additionally, the conceptual framework of reduction, refinement and replacement of animal experiments (CAAT Vision/Mission Statement) has prompted the present study aimed at the evaluation of the potential carcinogenic effect of CNC using pulmonary epithelial cells (BEAS-2B and A549). Long term repeated exposure of the cells to occupationally relevant sub-toxic concentration of CNC derived from wood (powder and gel) enhanced neoplastic-like transformation as demonstrated by increased cell proliferation, anchorage-independent growth, migration and invasion. Analysis of apoptosis revealed no effect in A549 and inhibitory effect of CNC in BEAS-2B cells. CNC exposure induced a strong activation of cells as demonstrated by the high number of cytoplasmic vacuoles, surface finger-like protrusions, lipid droplets and multi-nucleation. The signaling mechanisms of the cells were precisely defined by their cytokine responses. The increased proliferation was synergistic and mediated by both pro-inflammatory and pro-carcinogenic cytokines. The results of detailed analysis of cytokines were in line with their proliferative and transformative potential of the different CNC materials investigated in this study. Overall, our results provide novel information and evidence to suggest the potential carcinogenic effect of CNC.

Keywords: Green nanomaterials, cellulose, cells proliferation, migration, transformation, occupational safety

126. Specific Uptake Mechanisms of Well-Tolerated Thermoresponsive Polyglycerol-Based Nanogels in Antigen-Presenting Cells of the Skin

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Engineered nanogels are of high value for a targeted and controlled transport of compounds due to the ability to change their chemical properties by external stimuli. As it has been indicated that nanogels possess a high ability to penetrate the stratum corneum, it cannot be excluded that nanogels interact with dermal dendritic cells (DCs), especially in diseased skin. In this study the potential crosstalk of the thermoresponsive nanogels (tNGs) with the DCs of the skin was investigated with the aim to determine the immunotoxicological properties of the nanogels. The investigated tNGs were made of dendritic polyglycerol (dPG) and poly(glycidyl methyl ether-co-ethyl glycidyl ether) (p(GME-co-EGE)), as polymer conferring thermoresponsive properties. Although the tNGs were taken up, they displayed neither cytotoxic and genotoxic effects nor any induction of reactive oxygen species in the tested cells. Interestingly, specific uptake mechanisms of the tNGs by the DCs depended on the nanogels cloud point temperature (Tcp), which determines the phase transition of the nanoparticle. The study points to caveolae-mediated endocytosis as being the major tNG uptake mechanism at 37 °C, which is above the Tcp of the tNGs. At 29 °C, which is the Tcp of the tNG, beside caveolae-mediated endocytosis, macropinocytosis was involved, as well. In summary, our study highlights the impact of thermoresponsivity on the cellular uptake mechanisms which has to be taken into account if the tNG is used as a drug delivery system.

Keywords: Biokinetics/toxicokinetics, Genotoxicity, In vitro toxicology

127. Acute Toxicity of Boron Nitride Nanotubes In-Vitro and In-Vivo

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Boron nitride nanotubes (BNNTs) are an emerging engineered nanomaterial that has attracted significant attention due to its superior electrical, chemical, and thermal properties. As the number of applications grow, occupational exposures will likely increase. Since its toxicity is largely unknown, we performed acute in-vitro and in-vivo exposure studies with 13-23 nm diameter x 0.6-1.6 μm length BNNTs. THP-1 wild-type and NLRP3 knockout human monocytic cells were exposed to 0-100 μg/ml and male C57BL/6 mice were exposed by pharyngeal aspiration to 4 or 40 μg BNNTs and sacrificed 4 and 24 h post-exposure. The multi-walled carbon nanotube-7 (MWCNT-7) served as a particle control. In-vitro, BNNT induced a dose-dependent increase in cytotoxicity. This was confirmed in vivo by increased bronchoalveolar lavage levels of lactate dehydrogenase, a measure of lung cytotoxicity. In vitro, lysosomal destabilization, likely a result of particle uptake, was evident by acridine orange staining. Subsequent NLRP3 inflammasome activation was demonstrated by a dose dependent increase in IL-1β protein as well as cathepsin B and caspase 1 activity. Toxicity, and IL-1β production were attenuated in exposed NLRP3 knockout THP-1 cells. In-vivo, elevated pulmonary IL-1β confirmed in vitro findings. Consistent with suspected BNNT-induced inflammation, pulmonary neutrophil influx and inflammatory markers (Il6, Ccl2, Ccl22, Cxcl2) increased in a dose-dependent manner. In general, toxicity induced by BNNTs was less in comparison to MWCNT-7. Taken together, these results demonstrate that BNNTs induce inflammation in vitro and in vivo in part due to NLRP3 inflammasome activation and sub-chronic in vivo studies are warranted.

Keywords: Emerging nanomaterials

128. Toxicological Assessment of Shape-Engineered Titanium Dioxide Nanoparticles

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Beyond obvious contrasts in toxicity between different types of nanoparticles (NP), often also considerable differences in effects have been reported within a specific type of nanomaterial. The highly controlled generation and characterisation of a set of shape-engineered TiO_2 NP within the EU-FP7 project SETNanoMetro allows us to investigate the role of subtle shape- and surface structure changes (e.g. spheres, truncated bipyramids, platelets) on NP toxicity. Cytotoxicity, oxidative stress, pro-inflammatory and DNA damaging effects were evaluated in NR8383 rat alveolar macrophages and A549 human lung epithelial cells. All TiO_2 samples showed low toxicity (WST-1) towards both cell lines, in comparison to SiO_2 NP. In the A549 cells, several TiO_2 samples caused low levels of DNA strand breakage, but oxidative DNA damage induction was not detected (Fpg-comet assay). Interestingly, increased oxidative DNA damage could be observed for specific samples after photocatalytic activation with UV-B light, and this could be prevented by N-acetylcysteine. TiO_2 NP also caused minimal oxidative stress (e.g. HO-1, gamma-GCS) in the NR8383 cells. Whereas IL-6 mRNA expression in the macrophages was solely increased after SiO_2 treatment, several TiO_2 NP caused a significant induction of IL-1β mRNA expression. However, increased secretion of IL-1β protein by TiO_2 NP was only found from lipopolysaccharide-primed macrophages. Future aim of this study is to build and model nano-structure activity relationships for TiO_2 based on the developed physico-chemical database of the SETNanoMetro project. Supported by the EU Seventh Framework Programme.

Keywords: Genotoxicity, Hazard ranking/characterization, In vitro toxicology

129. 3D Airway Models Using the Air Exposure Route: First Steps Towards an In Vivo Replacement

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Human 3D airway models are fully differentiated and functional models of the respiratory epithelium and therefore may be positioned in safety evaluation of (nano)particles. Cultured at an air-liquid interface, they allow relevant exposure via air. MucilAirTM and A549 or Beas-2B cell cultures were exposed at ALI conditions in Vitrocell exposure modules to metal-oxide particles for 1hour. It was found that MucilAirTM cells were less affected by the air stream compared to both cell lines. Upon cerium-oxide exposure MucilAirTM cells induced only a mild oxidative stress response. In contrast the Beas-2B cell line showed an inflammatory and cytotoxic response. Both cell lines demonstrated a genotoxic response. Using cytokine and gene expression responses of MucilAirTM to copper-oxide particle exposures we show that the influence of the parameter ‘concentration’ is the largest, followed by ‘donor’, ‘unit’ and ‘session’, closed by ‘insert’. Finally MucilAirTM of healthy and asthmatic origin were exposed to copper-oxide particles. MucilAirTM cells of asthmatic origin were more affected by the exposure, showing a greater cytotoxicity response (LDH), but also showed a greater variation in this response. Summarizing, our results show that 3D MucilAirTM is more resistant to air stream and particles compared to 2D-cell cultures, most likely due to its in vivo relevant and protective morphology. Therefore, human 3D airway models might predict a more realistic response compared to 2D-cell cultures and can be used to assess the effects of particles, as long as donor- and session- variability are taken into account in the experimental design and subsequent statistical analyses.

Keywords: Cerium oxide nanoparticles, In vitro toxicology, Metal/metal oxide nanomaterials

130. Ferric Oxide Nanoparticles Induce Disruption of Iron Homeostasis and Production of ROS In Vitro

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Metal oxide nanoparticles (nMO), including ferric oxide (nFe_2O_3), can be used to great benefit in a wide variety of nano-technological applications. However, despite their rapid increase in production, adverse health consequences, particularly in an occupational setting, have yet to be thoroughly assessed. It is known that iron nanoparticle deposits are found in the lung macrophages of welders, and are thought to contribute to adverse respiratory outcomes. Previous research also suggests the potential carcinogenicity of nFe_2O_3 but with an unknown mechanism. Our previous research has shown that a sub-chronic exposure (up to 10 weeks) of nFe_2O_3 to primary human small airway epithelial cells induces cellular transformation. Based on observations that nFe_2O_3 may increase intracellular iron directly and/or disrupt iron homeostasis, we hypothesize that this influx of iron into the cell will participate in the Fenton reaction, thereby generating ROS, and ultimately resulting in DNA lesions and the observed cellular transformation. Following acute nFe_2O_3 exposure, our results show a significant change in expression of iron related proteins (import, storage, and export) with nFe_2O_3 treatment, as compared to non-treated or $nCeO_2$ treated cells. Acutely exposed cells also show an increase in intracellular iron, which corresponds to this flux in altered protein expression. Furthermore, nFe_2O_3 induces ROS production, while an amorphous SiO_2-coated nFe_2O_3 was protective against ROS production and observed cell death. These results indicate that changes in physicochemical properties of nFe_2O_3 may alter the bio-effects of this compound, and highlight the potential utility of “safe-by-design” strategies for preparing engineered nanomaterials.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Occupational safety, Toxicological mechanisms

131. Linkage between Noninvasive Electrophysiological Measurements Upon Exposure to Nanoparticles and Ultrastructural and Immunohistochemical Analyses

Karshak Kosaraju[1], Steven Crawford[2], Joseph Starobin[1], Shyam Aravamudhan[2]. [1]University of North Carolina at Greensboro; [2]North Carolina A&T State University, USA.

Although recent experimental findings have conclusively demonstrated the importance of biochemical and immunological markers for identifying nanoparticle-induced oxidative stress and vascular damage, the association of such exposure with noninvasive electrophysiological factors are not known. In this study, we investigated in vivo (a) the dynamics of novel cardiac repolarization based measure of reserve refractoriness (RoR) which reflects reserve of stability of propagation of excitation waves in the heart and (b) compared this novel method with conventional ultrastructural and immunohistochemical analyses. We found that mice exposed or instilled with ceria nanoparticles (60-100 nm) showed negative RoR for both low (20 µg/mouse) and high (200 µg/mouse) dosages with marginal statistical significance ($P > 0.064$). Immunohistological staining of lung tissue showed significant reduction of alveolar spacing even at low dosage and collapsed bronchioles at high dosage of ceria NPs. The ultrastructural TEM analysis showed that ceria NPs are distributed in both lung and heart, with NPs accumulated in lung causing in structural damage, as visualized in H&E staining. On the contrary, in the heart analogous structural damage of the tissue was not present, but presence of ceria NPs was still visible. Thus, one can conclude that changes in the reserve of refractoriness of the heart before and after NP exposure may be quite noticeable even in the absence of structural damage of heart tissue. The study of these effects under the influence of dobutamine stress is currently work in progress.

Keywords: Cerium oxide nanoparticles, In vivo toxicology, Metal/metal oxide nanomaterials, Occupational safety

132. Cellular Uptake and Solubility of Aluminum Nanoparticles

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Aluminum is the third most abundant element in the earth crust and therefore ubiquitously detectable in the environment. Mostly found in the form of derivatives such as silicates or oxides, it also occurs as metallic aluminum for example as colorant in sweets or in aluminum foil. With regard to potential toxicological effects, the different solubility of metallic aluminum nanoparticles compared to Al_2O_3 is of high relevance. Formation of ions may facilitate the crossing of blood-tissue barriers. Distribution in other organs with subsequent reformation of particulate aluminum due to milieu changes might occur. Therefore, the determination of solubility is required for proper risk assessment. Inductively coupled plasma mass spectrometry (ICP-MS) allows determination of aluminum with a detection limit of about 6 ppb. It could be proven that dissolution and solubility of metallic aluminum is significantly different when compared to Al_2O_3. Further analysis by time-of-flight secondary ion mass spectrometry (ToF-SIMS) revealed the uptake of aluminum and Al_2O_3 particles by proliferating and differentiated Caco-2 cells. For both particle forms common as well as different ions could be detected. In case of aluminum several aluminum-amino acid complex-derived ions from serine and valine were identified. For Al_2O_3, the main ions found were $Al_2O_2^+$, $AlOH^+$, AlH_2O^+ and $Al[(H_2O)_6]_3^+$.

Keywords: Metal/metal oxide nanomaterials, Methods, Physicochemical characterization

133. Localization and Distribution of Nanoparticles Generated During Real-Time Laser Material Processing in Rats

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Laser ablation is frequently used in the industry in material processing. Generated nanoparticles (NPs) [1] accumulate in the environment. The workers continously are exposed to those NPs. In animal studies, it has been shown that NPs lead to disruption of cellular signaling pathways and cellular toxicity [2,3]. However, it is not known whether NPs can gain access to systemic circulation of workers and accumulate in their organs in real life conditions. We investigated the body distribution of copper, tin and aluminum NPs (CuNP, SnNP and AlNP) generated in laser material processing environment similar to that of real life human exposure in rats. We have first characterized those NPs by SEM, TEM and EDX techniques. The rats were exposed to NPs for 3 months. For the first time, we have shown that exposure to NPs caused accumulation in various organs (such as lung, kidney, liver and spleen) during real time laser ablation process. Following SnNP exposure of rats, NPs up to 400 ppb were detected in lungs by ICP-MS (Figure 1). Chronic exposure of workers to NPs will result in end-organ accumulation and consequently may cause irreversible organ damage and serious health problems. This project is supported by TÜBİTAK (Project No: 113S223).

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Keywords: Biodistribution, Exposure assessment, In vivo toxicology, Metal/metal oxide nanomaterials, Occupational safety, Laser ablation, material processing, inhalation

134. Greener Synthesis of Anisotropic Nanoparticles using Luteolin Tetraphosphate

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Flavonoids occur naturally in many common fruits and vegetables. Previous studies show great results on flavonoids derived nanoparticles, but mostly are quercetin's derivatives. On the other hand; luteolin has been studied on its cancer inhibited and neurology characteristics. Both luteolin and quercetin have similar structures, with the only difference of extra carbonyl group on quercetin. Luteolin tetraphosphate (LTP) acts as both reducing and capping agent in synthesizing gold nanoparticles (AuNPs), and eventually silver nanoparticles. We hereby present a novel approach for the synthesis of gold nanoparticles (AuNPs) using water soluble, naturally-derived LTP. LTP was used as reducing agents and stabilizers. Synthesis was achieved at room temperature using water as a solvent and it requires no capping agents. The AuNPs were characterized using Uv-Vis, X-ray diffraction (XRD), Transmission electron microscopy (TEM), Energy dispersive absorption spectroscopy (EDS), High resolution transmission electron microscopy (HR-TEM) and selected area electron diffraction (SAED). The resulting AuNPs were spherical, triangular, cubicle, hexagonal and rectangular in shape. The AuNPs exhibited excellent antibacterial activities of 99.9%, 100% and 99.9% growth inhibition for Escherichia coli ATCC® 25922™, Staphylococcus epidermidis ATCC® 12228™ and Citrobacter freundii ATCC® 8090 at 104 cfu inoculations.

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Keywords: Biomedical/therapeutic applications, Environmental nanotoxicology, Exposure characterization, Green nanomaterials

135. END, an Annotated Nanomedicine Corpus

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Annotated corpora are a key resource for Natural Language Processing (NLP) and Information Extraction (IE) methods which employ machine learning. Although large-scale annotated corpora are available for pharmaceuticals, resources for nanomedicines are still limited. New engineered nanostructures and formulations are continuously being created, and NLP approaches can semi-automate the cataloguing and tracking of the different nanomedicines being developed. Today there is a critical need for use of NLP to extract knowledge and trends to build databases for nanotechnology research from an exponentially increasing body of literature. To support this aim, we have constructed a corpus of annotated drug product inserts taken from the U.S. Food and Drug Adminstration's Drugs@FDA online database. In this work, we present the development of the Engineered Nanomedicine Database (END) to support the evaluation of nano-entity extraction. The data was manually annotated for entity mentions consisting of nanomedicine physico-chemical characterizations, exposure information, and biological response information of over 40 FDA-approved nanomedicines. We present our experience in designing the annotation scheme. Experimental results are shown for inter-annotator agreement and comments are made on methodological considerations.

Keywords: Biomedical/therapeutic applications, nanomedicine, nanoinformatics

136. Integrative Functional Transcriptomic Analyses Implicate Specific Molecular Pathways in pulmonary toxicity from Exposure to Aluminum Oxide Nanoparticles

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Gene expression profiling has developed rapidly in recent years and it can predict and define mechanisms underlying chemical toxicity. Here, RNA microarray and computational technology were used to show that aluminum oxide nanoparticles (Al_2O_3 NPs) were capable of triggering up-regulation of genes related to the cell cycle and cell death in a human A549 lung adenocarcinoma cell line. Gene expression levels were validated in Al_2O_3 NPs exposed A549 cells and mice lung tissues, most of which showed consistent trends in regulation. Gene-transcription factor network analysis coupled with cell- and animal-based assays demonstrated that the genes encoding PTPN6, RTN4, BAX and IER play a role in the biological responses induced by the nanoparticle exposure, which caused cell death and cell cycle arrest in the G2/S phase. Further, down-regulated PTPN6 expression demonstrated a core role in the network, thus expression level of PTPN6 was rescued by plasmid transfection, which showed ameliorative effects of A549 cells against cell death and cell cycle arrest. These results demonstrate the feasibility of using gene expression profiling to predict cellular responses induced by nanomaterials, which could be used to develop a comprehensive knowledge of nanotoxicity.

Keywords: High throughput screening, pulmonary toxicology, aluminum oxide nanoparticle

137. Cellular Uptake Mechanism of Gold Nanoparticles with Three Different Surface Coatings and Protein Corona Effects on Cell Uptake in Human Epidermal Keratinocytes

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The surface charge and physicochemical properties of nanoparticles (NP) play an important role on protein corona formation and cellular uptake mechanisms. The kinetic cellular uptake from 15 min to 48h was studied in 40 nm and 80 nm gold NP (AuNP) with branched polyethyleneimine (BPEI), lipoic acid (LA) and polyethylene glycol (PEG) coatings in human epidermal keratinocytes. The uptake of AuNP with/without human plasma or human serum albumin (HSA) protein corona was quantified by ICP-MS and the cellular internalization pathways were investigated. BPEI-AuNP showed the greatest cellular uptake, whereas PEG-AuNP had the least with the highest value at 48h. Human plasma corona significantly decreased cell uptake of all AuNP, while HSA caused an even greater decrease compared to plasma. The mechanism of AuNP cell uptake was energy-dependent except for 40 nm LA-AuNP which depends on energy-independent membrane fusion and lipid raft-mediated internalization. BPEI-AuNP uptake was primarily through clathrin-mediated endocytosis and raft-mediated membrane fusion. Interestingly, plasma corona on 80 nm BPEI-AuNP significantly decreased cell uptake by inhibiting the endocytic pathways, while plasma corona on 40nm BPEI-AuNP had no effect. PEG-AuNP uptake was clathrin-mediated while plasma protein corona had no effect on the mechanism of uptake. These results suggest that the surface properties and the formation of a protein corona may significantly affect the AuNP cell uptake thus, providing a better understanding of the role of the protein corona on cellular uptake pathways. This information is important in the field of nanomedicine, cancer diagnosis, and treatment. Funding provided by the Nanotechnology Innovation Center of Kansas State.

Keywords: Biocorona, endocytosis, cellular uptake mechanism, gold nanoparticles

138. Nano-sized Elemental Selenium is Less Toxic and More Efficient in Capturing Mercury to Prevent its Accumulation and Transformation in rice

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Mercury (Hg) can concentrate in higher consumers from the food chain, and highly threaten the human health. To investigate the influence of nano-sized elemental selenium (nano-Se0) which has large specific surface characteristics on the uptake, accumulation and transformation of Hg in plants, garlic was exposed to different dosages of nano-Se0 and Hg^{2+} and analyzed after cultivation. The phytotoxicity of Hg with or without nano-Se0 addition was assessed. Distribution and speciation of Hg and Se were measured using synchrotron radiation based X-ray fluorescence (SR-XRF) combined with X-ray absorption near edge structure (XANES). It was found that nano-Se0 could efficiently mitigate the phytotoxicity of Hg and promote plant growth. SR-XRF and XANES analysis illustrated that nano-Se0 could largely capture Hg^{2+} to prevent it entering stele of root and translocation of Hg by forming Hg-Se compounds. Nano-Se0 inhibited Hg toxicity by promoting the conversion of Hg^{2+} to the less toxic Hg forms, such as RS-Hg-SR and R-Hg-R. The less toxic nano-Se0 shows great potential in prevention of Hg toxicity in food chain and has great implications for human health in Hg contaminated areas.

Keywords: Environmental nanotoxicology

139. Food Components in a Nanoparticle Digestion Model and Their Impact on Particle Uptake in Human Intestinal Cells

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Due to the increasing use of silver nanoparticles in food-associated consumer products, the oral uptake of silver nanoparticles has become a serious scenario. Two barriers must be considered regarding the question of NP fate: first, the strong shift in chemical conditions during digestion and second, the intestinal barrier which is mainly composed by enterocytes. Therefore, this study aimed to analyze changes in uptake of silver nanoparticles by using an in vitro digestion model. Food components were also implemented to simulate realistic conditions. Silver nanoparticles were digested in absence or presence of food components. All particle suspensions were analytically monitored by small-angel X-ray scattering (SAXS) during digestion. For transport studies, the TranswellTM-system was used. Particle uptake was determined by chemical digestion and AAS. The CTB assay was applied for cytotoxicity testing in the Caco-2 cells. Cytotoxicity testing revealed that neither the digestion process nor the presence of food components affected the cytotoxicity of the nanoparticles. Additionally, undigested as well as particles digested in the presence of food components were comparably taken up by intestinal Caco-2 cells, whereas the uptake of particles digested without food components was decreased by 60%. Our findings suggest that ingested nanoparticles may reach the intestine in a nanoscaled form. Apparently, it seems to play a crucial role if the particles are embedded in a food matrix during ingestion or not. This could lead to misinterpretation of uptake results. Therefore, digestion models and especially the presence of food components should be obligatory in validated test systems.

Keywords: Alternative testing methods/strategies, In vitro toxicology, Metal/metal oxide nanomaterials, Caco-2, Transwell, oral uptake model, small-angle X-ray scattering, food components, in vitro digestion

140. Quantification of Cellular Uptake of Silver Nanoparticles in Intestinal Cell Models of Various Complexities

Dajana Lichtenstein[1], Thomas Meyer[2], Linda Böhmert[1], Holger Sieg[1], Sabine Juling[1], Sören Selve[3], Andreas F. Thünemann[4], Irina Estrela-Lopis[2], Albert Braeuning[1], Alfonso Lampen[1]. [1]Federal Institute for Risk Assessment; [2]Institute of Medical Physics & Biophysics; [3]Technische Universität Berlin; [4]Federal Institute for Materials Research and Testing, Germany.

Several studies show that silver nanoparticles can reach the intestinal epithelia. Nevertheless, only sparse data concerning the direct quantification of cellular uptake of silver nanoparticles are available. Therefore, this study was focused on a systematical quantitative comparison of the cellular uptake of differently coated silver nanoparticles of comparable size. Intracellular uptake was determined quantitatively via a Transwell™-System with subsequent elemental analysis (AAS) and Ion Beam Microscopy (IBM). Silver nanoparticles were coated with poly (acrylic acid) and polyvinylpyrrolidone and Tween 20 and characterized extensively by TEM, DLS, SAXS, Zetasizer and NanoSight. AgPURETM as a widely used reference nanoparticle coated with Tween 20 and Tagat TO V was also used for comparison. Different intestinal cell models were applied to get closer to the complex in vivo situation: Beside the widely used intestinal Caco-2 model we also investigated an M-cell and mucus-model. Our findings suggest that silver uptake is clearly a particle-related effect. The internalization of silver nanoparticles was enhanced in uptake- specialized M-cells. Rutherford Backscattering Spectrometry (RBS) allowed distinguishing between adsorbed and internalized material and the results were in accordance with the Transwell™ data. Additionally, Particle-Induced X-Ray Emission showed intracellular association of silver with sulfur. We conclude that, the quantification of silver nanoparticle internalization revealed a clear particle-specific and a coating-related uptake. Furthermore, a high amount of silver nanoparticles is taken up in cell models of higher complexity. Thus, an underestimation of particle effects in vitro might be prevented by considering cell models with greater proximity to the in vivo situation.

Keywords: Dosimetry, In vitro toxicology, Metal/metal oxide nanomaterials, Caco-2, Transwell, Ion Beam Microscopy, Rutherford Backscattering Spectrometry, M-cells, Mucus

141. Impact of the Core and Coating Material on the Translocation of Nanoparticles In Vitro

Dajana Lichtenstein[1], Linda Böhmert[1], Holger Sieg[1], Thomas Meyer[2], Sabine Juling[1], Sören Selve[3], Andreas F. Thünemann[2], Irina Estrela-Lopis[4], Albert Braeuning[1], Alfonso Lampen[1]. [1]Federal Institute for Risk Assessment; [2]Institute of Medical Physics & Biophysics; [3]Technische Universität Berlin; [4]Institute of Medical Physics & Biophysics, Germany.

Nanoparticles are becoming increasingly important role in consumer-related products. Understanding the interactions between nanoscaled objects and living cells is therefore of great importance for risk assessment. In this context, it is generally accepted that nanoparticle size and shape are crucial parameters regarding the potential of nanoparticles to penetrate cell membranes and epithelial barriers. Current research in this field additionally focuses on the particle coating material. In order to distinguish between core- and coating-related effects in nanoparticle uptake and translocation behavior, this study investigated two nanoparticles equal in size, coating and charge but different in core material. Silver and iron oxide were chosen as core materials. Nanoparticles were coated with poly (acrylic acid) and characterized by TEM, SAXS, ZetasizerTM and NanoSightTM. For uptake and transport studies the human intestinal Caco-2 model in a TranswellTM-system with subsequent elemental analysis (AAS) was used. For particle visualization TEM and Ion Beam Microscopy (IBM) were conducted. Although similar in size, charge and coating material, the behavior of particles in Caco-2 cells was quite different. The internalized amount was comparable, but PAS-coated iron oxide nanoparticles were additionally transported through the cells. By contrast, PAS-coated silver nanoparticles remained in the cells. Our findings suggest that the coating material influenced only the uptake of the nanoparticles whereas the translocation was determined by the core material. In summary, a core-dependent effect on nanoparticle translocation was revealed. Both the uptake and transport of nanoparticles in and through cells should be considered when discussing nanoparticle fate and safety.

Keywords: In vitro toxicology, Caco-2, Transwell, silver nanoparticles, iron oxide nanoparticles, Ion Beam Microscopy

142. Toxicity of Engineered Silver Nanoparticles (NPs) is Enhanced by Co-Exposure with Clay Particles in Zebrafish Larvae

Corin Liddle, Steve Renton, Helinor Johnston, Theodore Henry, Teresa F Fernandes. Heriot-Watt University, UK.

Naturally occurring particulates within the nano-size range are very abundant in surface waters and among these are mineral clay particles. Engineered nanoparticles (NPs) that are released into surface waters will undoubtedly interact with naturally occurring particulates; however, investigations of the effects of these interactions on NP toxicity have not been conducted. Clay minerals consist of silica tetrahedral (T) and alumina octahedral (O) layers in a lamellar structure that results in patch-wise surface heterogeneity with both positive and negative charges on their surfaces. The presence of both polarity charges on the surface of clay particulates suggests that interactions with both negatively charged silver (Ag) NPs and dissolved free silver ions (Ag+) will occur. We hypothesised that interactions between Ag-NPs and clays would alter aqueous phase Ag-NP toxicity in zebrafish Danio rerio larvae (ZFL). Parallel Ag-NP (0-3 mg/l) suspensions were prepared with and without 20 mg/l of Kaolinite clay suspension, and zebrafish larvae were exposed for 96 h (age 72-168 h post fertilization). Consecutive experiments were conducted with increasing concentrations of clay and humic acid (0-100 mg/l) and fixed concentrations of Ag-NPs (0.75, 1, 1.5 and 2 mg/l), and mortality of larvae was recorded. The clay exposed controls showed no toxicity from clay alone. Mortality of ZFL significantly increased when fish were exposed to both Ag-NPs and clay. The toxicity of 1 mg/l Ag-NPs increased with clay concentration by up to a maximum increase of 0.4 fold with 60 mg/l of clay, relative to 1mg/l of Ag-NP only. The 96-h Ag-NP exposure concentration predicted to kill 50% (LC50) of ZFL with and without clay (20 mg/l) was 0.73±0.1 and 1.1±0.1 mg/L (±SEM) respectively, a 0.66 fold increase in toxicity within the clay and Ag-NP co-exposure. Humic acid significantly decreased toxicity of Ag-NPs (consistent with previous reports); however, the presence of humic acid did not eliminate the increased toxicity of Ag-NPs when co-exposed with clay. The observed increased toxicity of Ag-NPs in ZFL when co-exposed with clay is environmentally relevant and a novel result that suggests an alternative facet regarding the environmental effects of engineered nanoparticles.

Keywords: Environmental nanotoxicology, silver nanoparticles, coatings, clay, fate, realistic environmental conditions, zebra fish

143. Assessing Aqueous Phase Silver Nanomaterial (Ag NM) Environmental Fate: A Flume Based Approach

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Heriot-Watt University, UK.

Flumes offer the ability to study Ag NM removal and persistence behaviour in controlled and potentially 'environmentally relevant' scenarios. Studying removal rates within flow systems may produce fate descriptors to translate processes into useful models which will help with exposure assessment in realistic conditions. The aim of this research was to develop flume based systems and methods to investigate aqueous phase Ag NM fate and behaviour within different aquatic conditions, and subsequently investigate the effects of flow, organic and inorganic matter, the presence of non-cohesive sediments and re-suspensions events on aqueous phase Ag NM removal/persistence and re-dispersion. Two Ag NM were studied within flume systems (flow velocity 0.12 m/s): Ag NM (PVP) (30-50nm), and analogous reference material NM300 (15±5nm), within two recirculating turbulent flow test systems containing 1.8 L and 55 L of soft water medium, respectively. Studies were carried out over a time course of up to 24 days. At the end of the experiment flow velocity was increased to entrain the bed sediment and create re-suspension events. Suwannee river humic acid (SRHA), Sigma humic acid and kaolinite clay were used as model organic and inorganic matter, and silica sand was also used as a model non cohesive sediment. Results indicated NM300 removal rates and aggregation state within the aqueous system increased within turbulent flow relative to static systems, indicating turbulent flow significantly increased removal processes, which was attributed to increased sedimentation rates. The addition of kaolinite clay (20 mg/l) and humic acid (2 mg/l) did not significantly increase or stabilise Ag NM aqueous phase removal within flow conditions. However higher concentrations of humic acid (10 mg/l) resulted in longer term persistence of NM300 within the water column. The presence of non-cohesive sediments significantly increased removal rates of NM300. Ag NMs exhibited re-dispersion following re-suspension events. These results indicate that turbulent flow would be the dominating factor in modulating Ag NM removal and remobilisation in lotic water, when organic matter concentrations are low.

Keywords: Environmental nanotoxicology, silver nanoparticles, coatings, turbulent flow, fate, humic acid, organic matter, realistic environmental conditions

144. Green Nanomaterials: All about Fusion

Igor Linkov, Matthew Bates. US Army Engineer Research and Development Center, USA.

An inherent requirement of ‘green’ nanomaterials includes a fusion of green chemistry and nanotoxicology. Where fusion is generally understood as "the process or result of joining two or more things together to form a single entity", a central question pertaining to green nanomaterials centers on how to fuse unique scientific disciplines of chemistry and toxicology into a single entity remains unresolved. Early examples of attempts to resolve this issue by integrating known factors of nanomaterial toxicology have been limited in focus, such as with coating of quantum dots and the study of environmental, health, and safety concerns of resultant nanomaterials AFTER manufacturing. Within such an exercise, fusion is at best used colloquially to visualize individual metrics of nanomaterials without a serious attempt to ‘form a single entity’ of a true green nanomaterial. To begin to shift discussion of how such fusion is undertaken, we argue that factors derived from chemistry and toxicology may be combined if technical data is connected with expert judgment, which would be used to facilitate optimal nanoproduct or nanomaterial design BEFORE it actually is manufactured. Within this meeting, tools of Multi-Criteria Decision Analysis will be presented as a foundation for such integration. Case studies pertaining to MCDA will be discussed.

Keywords: Green nanomaterials, Regulatory decision making, Risk assessment

145. Risk-Based and Prevention-Based Governance for Nanotechnology

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The emergence of new materials and technologies (e.g., nanomaterials, synthetic biology) pose significant challenges to regulatory bodies. Risk governance includes rules conventions, processes and mechanisms by which decisions about risks are taken and implemented. For decades, risk governance adopted a conventional risk management approach in which threats and hazards are quantified in absolute units (e.g., cancer risk, probability of system failure) and controlled to acceptable levels. The conventional risk management approach can be effective where (i) threats are well understood, (ii) the set of potential adverse outcomes is known, and (iii) the respective probabilities of their occurrence can be quantified. However, where any of these three conditions are missing, a different approach to risk governance is required in order to facilitate the development of beneficial technologies. One potential alternative is prevention-based governance. Prevention-based governance seeks to avoid or minimize threats, vulnerabilities, and consequences by mandating, directly incentivizing, or encouraging the adoption of inherently safer alternative materials, technologies and systems. Although regulatory agencies have expressed a preference for prevention over control for decades, the notion of prevention has rarely been incorporated into mainstream enforceable regulation or governance more broadly. We argue that developing decision analytic tools to guide risk governance would make prevention-based risk governance for nanotechnology practical and achievable.

Keywords: Hazard ranking/characterization, Regulatory decision making, Risk assessment

146. The Toxicity of Differently Coated Copper Oxide Nanoparticles Towards the Marine Blue Mussel, Mytilus Edulis

Simon Little, Mark Hartl, Teresa F Fernandes. Heriot-Watt University, UK.

Rapid growth in the field of nanotechnology is continually increasing the potential release of nanoparticles (NPs) into the environment. Although copper oxide nanoparticles (CuO NPs) are widely used within numerous industrial and commercial applications (e.g. batteries, electronic circuits, superconductors, solar energy conversion and gas sensors), their toxicity is still poorly understood in comparison with other metal oxide NPs. As the main recipients of industrial and domestic wastewaters, the aquatic environment will inevitably become a sink for CuO NPs throughout their life cycle. Filter-feeders, such as the blue mussel, (Mytilus edulis) are widely used in environmental monitoring and are organisms of great interest in regards to the fate and toxicity of NPs within the aquatic environment. This study, funded by the FP7 project, NANOSOLUTIONS, aims to investigate the mode of toxicity exhibited by CuO NPs towards M. edulis and the effect of surface coatings upon CuO NP toxicity. M. edulis were exposed to core CuO NPs and CuO NPs coated with polyethylene glycol, nitrate and carboxylic acid, for 48 hours at concentrations of 10 and 20 $\mu g/L^{-1}$. Following exposure, the activity of the antioxidant enzyme superoxide dismutase (SOD) was assessed in M. edulis gill and digestive gland tissues to determine whether reactive oxygen species (ROS) were formed as a result of CuO NP exposure, whilst the TBARS (Thiobarbituric Acid Reactive Substances) assay was used to determine whether lipid peroxidation (a product of oxidative stress) had occurred. Additionally, ASCGE (alkaline single cell gel electrophoresis) comet assays were performed on gill and haemolymph cells to assess potential DNA damage caused by CuO NP exposure. It was hypothesised that an increase in CuO NP concentration would lead to an increase in M. edulis SOD activity, lipid peroxidation and DNA damage, whilst coatings would influence CuO NP toxicity. Work is currently underway to test these hypotheses.

Keywords: Environmental nanotoxicology, Mytilus edulis, marine mussel, copper oxide nanoparticles, coatings, genotoxicity, oxidative stresseprotoxicity

147. Combinatorial Toxicity of Zinc Oxide and Titanium Dioxide Nanoparticles in Human Primary Epidermal Keratinocytes

Joachim Loo, Ng Kee Woei, Mustafa Kathawala. Nanyang Technological University, Singapore.

Nanoparticles have been a subject of intense safety screenings due to their influx in various applications. Although recent studies have reported on the plausible cytotoxicity effects of nanoparticles, many of these focused only on single material nanoparticles, while the cytotoxicity effects of dual nanoparticle systems (e.g. ZnO with TiO_2) has remained unexplored. For example, commercial products like sunscreens and cosmetics contain both nano-sized ZnO and TiO_2, but dual nanoparticle cytotoxic studies are meager. In this submission, the cytotoxicity of a dual particulate system comprising both ZnO and TiO_2 was evaluated in vitro on skin-mimicking human primary epidermal keratinocytes (HPEKs). Results revealed that ZnO nanoparticles were partially soluble (up to 20 μgml⁻1 after 1 day) and could induce strong cytotoxicity as compared to the insoluble TiO_2 nanoparticles which remained non-toxic until very high concentrations. It was found that TiO_2 nanoparticles could play “vigilante” by protecting keratinocytes from acute toxicity of ZnO nanoparticles, as TiO_2 nanoparticles were found to adsorb and immobilize free Zn2+ ions. This study reveals a unique dual-nanoparticle observation in vitro on HPEKs, and highlights the importance of dual nanoparticle system toxicity studies, especially in applications where more than one nanoparticle material-type is present. This work was supported by the School of Materials Science and Engineering (M020070110), Nanyang Technological University.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Physicochemical characterization

148. A Study to Assess the Ability of Different In Vitro Exposure Models to Predict In Vivo Pulmonary Adverse Effects

Thomas Loret[1], Emmanuel Peyret[1], Benedicte Trouiller[1], Anne Braun[1], Christophe Egles[2], Ghislaine Lacroix[1].
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Inhalation is a major exposure route for metallic and low soluble nanomaterials (NM), and TiO_2 is one of the most used in nanotechnology products. The aim of our study is to evaluate if using more complex in vitro methods, by exposing alveolar cells at the air-liquid interface (ALI) to nano-TiO_2 aerosols, gives more predictive toxicological results compared to submerged exposures to suspensions. Three different nano-TiO_2 (NM105, NM101, NM100) were used. An exposure system was set up using Vitrocell® devices to expose cells at the ALI during 3h. Cells (A549 or A549+THP-1) were exposed at the ALI to aerosols or in submerged conditions to NM suspensions. Rats were exposed by intratracheal instillation. Biological activity (viability, oxidative stress and cytokines) was assessed at 24h, in vitro on cells and in vivo on bronchoalveolar lavage fluids, to perform vitro/vivo comparisons. The doses achieved using our in vitro aerosol exposure system (maximum around 3 µg/cm²) were sufficient to observe biological effects on the coculture. Generally, we observed effects at lower doses when exposing cells to aerosols compared to suspensions. In vivo experiments are ongoing. For now we observed that the NM101 seems to induce more biological adverse effect than the NM100 both in vivo and in vitro. Further comparisons between in vivo and in vitro results will be performed to assess the predictivity of the in vitro methods used. This work is supported by the French ministry of environment (“Programme 190”) and by the EU-FP7 (NANoREG project).

Keywords: Alternative testing methods/strategies, In vitro toxicology, In vivo toxicology, Metal/metal oxide nanomaterials

149. Sustainable Nanoscale Synthesis and Antibacterial Activities of Gold Nanoprisms derived from Apigenin Triphosphate

David Luther, Francis Osonga, Omowunmi Sadik. SUNY Binghamton University, USA.

The concept of sustainable nanotechnology involves the nanoscale control of synthesis and processing of matter without footprints that give rise to environmental degradation. Hence there is a search for synthetic methods that utilize fewer amounts of materials, water, and energy; while reducing or replacing the need for organic solvents. Herein we analyzed synthetic methodology of gold nanoparticles (AuNPs) using the flavonoid apigenin, an organic compound found naturally in chamomile and parsley, among other places. While the nanosynthetic properties of apigenin have been well-documented in the past, those of phosphate derivatives of apigenin such as apigenin triphosphate (ATRP) have not. The modified physio-chemical properties of derivatized apigenin grant the compound significantly modified reactivity, and the capability for complexation using only water as a solvent. This provides a possible method for 'greener' nanoparticle synthesis, by minimizing the usage of toxic organic solvents. Our characterization of the synthetic conditions for ATRP and apigenin-based AuNPs incorporated numerous analytical techniques, including UV-visible and fluorescence spectroscopy to monitor the characteristic AuNPs peak at ≈535 nm over time and with varied kinetic reaction conditions (temperature, concentration, pH), FTIR spectroscopy to characterize present functional groups, and DLS/TEM analysis to discover AuNPs of novel shapes, including triangular and hexagonal conformations, and of average size 5-25 nm AuNPs exhibited excellent antibacterial activities with inhibition of over 99% was found in C. freundi, Staphylococcus epidermidis and E. Coli.

Keywords: Environmental nanotoxicology, Green nanomaterials, Metal/metal oxide nanomaterials, green synthesis, antibacterial

150. Where Does the Transformation of Precipitated Ceria Nanoparticles in Hydroponic Plants Take Place?

Yuhui Ma, Zhiyong Zhang, Peng Zhang. Institute of High Energy Physics, Chinese Academy of Sciences, P.R. China.

Cerium oxide nanoparticles (CeO_2 NPs) have been found to be partly biotransformed from Ce(IV) to Ce(III) in plants, yet the transformation process and mechanism are not fully understood. Here we try to clarify the specific site and necessary condition for the transformation of precipitated CeO_2 NPs in hydroponic cucumber plants. Three different treatment modes were adopted according to whether the NPs were incubated with roots all the time or not. Results showed that exposure modes significantly affect the translocation and transformation of CeO_2 NPs. In the normal exposure mode, Ce was present as Ce(IV) and Ce(III) mixture in the roots and shoots, and the proportion of Ce(III) in the shoots was enhanced obviously with the increase of exposure time. The results of short-time incubation and petiole exposure modes suggested that CeO_2 NPs could not be reduced within a short incubation time (3 h) or be further reduced inside the plant tissues. It was deduced that root surfaces are the sites and the physicochemical interaction between the NPs and root exudates at nano-bio interface is the necessary condition for the transformation of CeO_2 NPs in plant systems. These results will contribute to understand the transformation mechanism of CeO_2 and other metal-based NPs and properly evaluate their ecological effects.

Keywords: Environmental nanotoxicology

151. Metal Oxide Nanoparticle Ingestion alters Intestinal Nutrient Absorption and Enzyme Activity

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Overall well-being is related to gut health and function, and the gastrointestinal (GI) tract serves as a critical interface between the body and the external environment. Nanoparticles (NPs) enter the food chain from products such as processed foods and medications; which makes ingestion nearly unavoidable. Americans consume 1012-1014 engineered fine to ultrafine particles per day, primarily from metal oxides such as TiO_2, SiO_2, and ZnO. The goal of our work is to use an in vitro cell culture model of the GI tract to examine how NPs widely used in food affect intestinal functionality, including nutrient absorption and brush border enzyme activity. Physiologically relevant acute (four hours) or chronic (five days) doses of 30 nm TiO_2, 30 nm SiO_2, or 10 nm ZnO in culture medium or chyme mimic significantly altered iron, zinc, and glucose transport. Intestinal alkaline phosphatase, which regulates the absorption of fatty acids across the apical intestinal epithelial membrane, was also affected by NP exposure. These changes were not due to alterations in tight junction functionality. Gene expression analysis showed that NPs change the level of nutrient import protein gene expression. The changes in nutrient transporter expression could be related to ROS production or pro-inflammatory signaling relevant to the intestinal epithelium. Overall, these results suggest that intestinal epithelial cells are affected at a functional level by physiologically relevant exposure to NPs, and that the cells are working to regulate the transport mechanisms disturbed by NP ingestion. This work was supported by the National Institutes of Health (1R15ES022828).

Keywords: Human toxicology, In vitro toxicology, Metal/metal oxide nanomaterials, nanoparticle ingestion, GI tract

152. Potential Inhalation Exposures to Particles from Nanotechnology-Enabled Clothing

Gedi Mainelis, Leonardo Calderon, Letao Yang, KiBum Lee. Rutgers University, USA.

This study analyzed potential release of particles from 11 nanotechnology-enabled clothing items containing silver nanoparticles. The TEM was used to investigate the presence and size of nanoparticles in the items. The concentration of metals was determined using ICP-MS and ashing. The potential release of particles into the air during clothing wear was simulated by using a rotary abraser (Taber Industries Inc.) with felt abrading wheels. Experiments were performed with brand new items as well as items that have been washed multiple times to simulate their natural wear and tear. The released particles were measured using a Scanning Mobility Particle Sizer and an Aerodynamic Particle Sizer (TSI Inc.). As per TEM, nanoparticles were found in most investigated products, but their size and abundance depended on a product. The concentration of silver in investigated clothing samples varied from 50 ppb to tens of ppm. Abrasion experiments released particles ranging from 3 nm to 20 µm in diameter with total concentration ranging from $10^2/cm^3$ to $10^4/cm^3$. After washing, some products released higher particle concentrations while others released lower particle concentrations. The released particles were investigated for presence of silver by capturing them on TEM grids and analyzing via TEM/EDX. This results shows that the use of investigated nanotechnology-enabled clothing could result in the release of and potential inhalation exposure to silver nanoparticles.

Keywords: Exposure assessment, Nanomaterial release, Consumer products

153. Unlike their Bare Analogs, PEGylated Silver Nanoparticles Acquire Diverse Coronas, but do not Translocate to Systemic Immune Cells

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Nanoparticles decorated with polyethylene glycol (PEG) coatings diffuse readily in mucus, unlike their “bare”-counterparts. Because mucus is one of the most significant biological barriers between the environment and lung epithelium, it was previously suggested that inhaled PEG-nanomaterials are more likely to translocate systemically. Additionally, PEGylated nanoparticles are known to be less prone to acquiring biomolecular corona due to their “stealth” behavior.Herein we report somewhat paradoxical findings demonstrating that model 110 nm PEGylated silver nanoparticles (PEG-AgNPs) are found in negligible amounts in circulating immune cells after intrapharyngeal instillation in C57BL/6 mice, as analyzed by mass cytometry (CyTOF). In contrast, bare AgNPs reached systemic circulation and were found in $CD11b^+$ (4.2%) and $CD11c^+$ monocytes (0.52%), as well as $Ly6G/C^+$ neutrophils (3.1%), as shown in Figure 1A. The biomolecular corona of PEG-AgNPs formed after in vitro incubation with bronchoalveolar lavage fluid (BALF) was found to contain 141 unique proteins vs. 93 proteins in AgNPs. In addition, PEG-AgNP induced more prominent protein oxidation (vs. AgNP) as assessed by shotgun proteomics, which found at least 32 oxidation-sensitive proteins among 74 peptides containing terminal cysteine oxidation (Figure 1B), and 26 peptides containing tyrosine protein nitration (not shown).Our data suggests that PEG-AgNPs may acquire diverse protein coronas due their facile penetration in depths of the lung mucus layer. In this layer, they have access to oxidation-sensitive proteins, which, once oxidized, mediate the engulfment of the nanoparticles via surface-displayed oxidized engulfment cues. Thus, corona-mediated pathways of cellular internalization of nanoparticles warrant future investigation.

Keywords: Biocorona, In vivo toxicology, Metal/metal oxide nanomaterials, Proteomics, Mass-cytometry

154. Biomolecular Interactions of Emerging Two-Dimensional Materials with Aromatic Amino Acids

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The present work experimentally investigates the interaction of aromatic amino acids, viz., tyrosine, tryptophan, and phenylalanine with novel two-dimensional (2D) materials including graphene (G), graphene oxide (GO), and boron nitride (BN). Photoluminescence, micro-Raman spectroscopy and cyclic voltammetry were employed to investigate the nature of interactions and possible charge transfer between 2D materials and amino acids. Consistent with previous theoretical studies , graphene and BN were observed to interact with amino acids through π-π interactions. Furthermore, we found that GO exhibits strong interactions with tryptophan and tyrosine as compared to graphene and BN, which we attribute to the formation of H-bonds between tryptophan and GO as shown theoretically in previous studies. On the other hand, phenylalanine did not exhibit much difference in interactions with G, GO, and BN.

Keywords: Biocorona, Carbon-based nanomaterials, Emerging nanomaterials, Exposure assessment

155. Airborne Nanoparticle Release and Toxicological Risk from Metal Oxide-Coated Textiles: Toward a Multi-Scale Safe-By-Design Approach

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Nano metal oxides have been proposed as alternative to AgNPs for antibacterial coatings. Here polyester fabric has been sonochemically coated with nZnO, nCuO, and Zn-doped CuO (nZn-CuO). NPs with different size and shape have been collected. To understand the respiratory impact, the coated textiles have been subjected to abrasion tests and the generated airborne particles measured. The cytotoxic and pro-inflammatory effects have been investigated in human alveolar A549 and macrophage-like THP-1 cells exposed to the NPs. Reduced cell viability and increased interleukin (IL8) release already occur after 3 h exposure at high NP concentrations. The 24-h EC50 of nZnO and nCuO for both cell lines was 20-30 µg/ml. Differently from nZnO, nCuO induced IL8 release already at sub-toxic concentrations. No clear difference in the cell responses can be associated to NP shape and size. The effects of the most effective antibacterial nZn-CuO were comparable to those of nCuOs. Very little amount of particles in the airborne form have been detected after abrasion. Real-time measurement in the size range of 12 – 560 nm showed no obvious difference between the abraded particles and the background. The release of particles in the micrometer range was detected with the peak size around 1 – 2 µm, but the number concentration was low, in the range of hundreds of particles per cm3 or less. ZnO-coated samples seemed to release more particles than the nCuO-coated ones. In summary, these NMs should be considered safe in their application. This work was supported by Fondazione Cariplo. (OverNanotox 2013-0987).

Keywords: Composite nanomaterials, Environmental nanotoxicology, Exposure assessment, Metal/metal oxide nanomaterials

156. The Influence of Surface Functional Coating on the Silver Nanoparticle-Induced Reproductive and Developmental Toxicity

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Since their antimicrobial properties, Silver nanoparticles (AgNPs) are among the most exploited nanomaterials in consumer products. Size and surface reactivity and coating are critical physicochemical properties responsible for NP toxicity. In this study the reproductive and developmental toxicity of citrate- (Cit-) and branched polyethyleneimine- (BPEI-) coated 10 nm AgNPs was investigated on Xenopus laevis fertilization and embryos by the Frog Embryo Teratogenesis Assay-Xenopus (FETAX). The effects were compared also with the exposure to Ag ions. Light, confocal and electron microscopy analyses have been performed on sperms and embryos to characterize the NP-cell interactions and uptake, as well as the histological and ultrastructural lesions. The results suggest that the positively-charged BPEI-AgNPs are more effective in inducing adverse reproductive and developmental outcomes, likely as a consequence of the enhanced interactions with sperm membrane and embryonic barriers (e.g. fertilization envelope and intestine) in respect of the negatively-charged Cit-AgNPs. Although Ag^+ resulted strongly embryolethal, the BPEI-AgNPs showed the highest teratogenic index, pointing out the role of NP size and functional coating in determining the teratogenicity. No lethal and slight malformation effects were induced by Cit-AgNPs. We conclude that possible reduced fertility and teratogenic risks may be associated to BPEI-AgNPs exposure, but the modality of NP-tissue interactions and the teratogenic mechanism need further scrutiny to be defined. This work was supported by Fondazione Cariplo (OverNanotox 2013-0987).

Keywords: Alternative testing methods/strategies, Developmental nanotoxicology, Environmental nanotoxicology, Toxicological mechanisms

157. Surface Bio-Interactions and Oxidative Stress Stand at the base of the Very Early Nano Cuo-Induced Cell Toxicity in Lung Cells

Paride Mantecca[1], Elisa Moschini[1], Ilana Perelshtein[2], Aharon Gedanken[2], Graziano Colombo[3], Isabella Dalle Donne[3], Tommaso Serchi[4]. [1]Dept Earth and Environmental Sciences and [3]Department of Biosciences, University of Milan-Bicocca, Italy; [2]Department of Chemistry and Nanomaterials, Bar-Ilan University Center for Advanced Materials and Nanotechnology, Israel; [4]Department of Environmental Research and Innovation, Luxembourg Institute of Science and Technology, Luxembourg.

Copper oxide nanoparticles (nCuO) are heavily toxic in vitro. The mechanism of toxicity was related to oxidative insults, coming from dissolved copper ions and leading to apoptotic or autophagic cell death. We hypothesized that early oxidative events coming from specific NP surface reactivity are able to mine the cell integrity, driving to death. nCuOs with different structure and oxidative potential, were tested on A549 cells for 1h and 3h. Cells were analysed for viability and oxidative change of the proteome. Oxidative by-products were studied by immunocytochemistry and cell-NP interactions by confocal and electron microscopy. Significant oxidative changes were induced soon after 1h, as revealed by the increased protein carbonylation and thiol reduction. Stronger effects were observed for NPs with more defective crystalline structure. NPs interacted with cell surface and were taken up by small endocytic vesicles, but no dissolution was visible inside lysosomes, confirming the specific NP surface-dependent oxidative injury, culminating in the apoptotic program activation. These results introduce new paradigms for the toxicity of metal-based NPs, beyond the lysosomal-enhanced Trojan horse mechanism. The effects were investigated also in 3D co-cultures grown at the Air Liquid Interface and mimicking the alveolar-blood barrier.They resulted in principle more resistant, suggesting their use as a more realistic model for respiratory toxicology. This work was supported by Fondazione Cariplo (OverNanotox 2013-0987)

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

158. 90-day Repeated Dose Oral Toxicity Study on Synthetic Amorphous Silica NM203: the EFSA Approach

Francesca Maranghi, Laura Narciso, Sabrina Taitl, Andrea Martinelli, Antonio Di Virgilio, Mauro Valeri, Roberta Tassinari. Istituto Superiore di Sanità, Italy.

In the frame of NANoREG project, a repeated-dose 90-day oral toxicity study was carried out (OECD TG 408), suggested by EFSA for the hazard identification of nanomaterials relevant for food safety. The test material was amorphous silica dioxide (SiO_2 NM-203, JRC repository) - the food additive E551 - tested in the Nanogenotox Joint Action for toxicokinetic. Characterization and dispersion analysis of NM203 were performed using Nanogenotox protocols. Sprague Dawley rats (both sexes) were orally treated with 0 (vehicle only – milliQ water), 2, 5, 10, 20 and 50 mg/kg bw a day by gavage, 5 days/week for 90 days. Tissues and blood samples from control and treated rats were shared among national and international working groups as follow: animal experiment/Histopathological analysis/Serum biomarkers/Germ cell genotoxicity/characterization NM203 dispersion/biodistribution-bioaccumulation in target tissues (ISS Dpt. of Veterinary Public Health and Food Safety); Reproductive toxicity (Tor Vergata University of Rome); Genotoxicity male rats, male reproductive toxicity (ENEA, Italy); Genotoxicity female/male rats (ANSES, France and ISS Dept. Environment and Primary Prevention); Immunotoxicity (ISS Dpt. of Infectious, Parasitic and Immune-mediated Diseases). The results were used to identify a Benchmark Dose lower confidence limit. The NOAEL approach was used for regulatory purposes. NM-203 caused no signs of overt toxicity in both sexes, at any dose levels. Male rats showed a wider spectrum of effects in comparison to females. It was evident the difficulty of defining a clear dose-response relationship; different mode of actions can be hypothesized taking into account the dose levels. NANoREG - (FP7/2007-2013) GA 310584.

Keywords: Genotoxicity, In vivo toxicology, Risk assessment, Toxicological mechanisms, Food safety

159. Supporting Risk Assessment of Nanomaterials with Quality-Approved Information - Dana Literature Criteria Checklist

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Nanotechnology is of increasing significance for many sectors of industry opening the market for numerous new applications ranging from electronics to the health care system. Besides their great innovative potential, the large variety of existing synthetic nanomaterials used in the last decade together with all current and future new (nano)materials represents a major challenge for scientists and regulators in terms of measuring and assessing the potential hazard caused by the materials or the products themselves. Addressing the issue of material characterisation being one of the key challenges any nanosafety assessment and safe-by-design approache, the DaNa project team (Data and knowledge on nanomaterials, www.nanoobjects.info) developed and recently updated its' Literature Criteria Checklist providing the nanosafety community with a great tool for quality evaluation and management of scientific publications. This checklist includes mandatory and desirable assessment criteria covering the topics physico-chemical characterisation, sample preparation and necessary (biological) testing parameters ensuring a thorough, comprehensive and fit-for-purpose assessment of the used nanomaterial in any given setting (products, humans, environment). With this approach, the international DaNa-expert team has been processing scientific publications on nanomaterials dealing with safety issues for humans and the environment. All positively evaluated literature is then fed into the DaNa Knowledge Base and published on the website www.nanoobjects.info. This web platform offers easy-to-understand, up-to-date and quality-approved information on 26 market-relevant nanomaterials concerning their effects on safety of humans and the environment. DaNa2.0 is a national project funded by the German Federal Ministry of Education and Research (FKZ 03X0131).

Keywords: Hazard ranking/characterization, Methods, Risk assessment, quality assessment for nanotoxicology publications

160. Occupational Exposure to Nanoparticle Emissions in Commercial Photocopy Centers and the Case for a New IAQ Guideline

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Background: Virtually all hardcopy devices (HDs) (laser printers/photocopiers) use nano-enabled toners and emit nano-particles ($PM_{0.1}$) in great quantities; 700X indoor backround, and greater than most urban environments. $PM_{0.1}$ emissions universally include several transition metals (iron, zinc, titanium, chromium, nickel and manganese), organic carbon, and ENMs that can be traced directly to the toner. In-vitro studies suggest HD emissions induce DNA damage, oxidative stress, ROS, and have toxic potency equivalent or greater than welding fume. Exposure studies with healthy volunteers mirror these findings. There is little user awareness or control of the hazard, and no guidelines protecting workers or patrons from overexposure. Objective: To survey the photocopy work environment with regards to emissions controls and to evaluate IAQ with emphasis on $PM_{0.1}$ exposure(s) at the workstation. Methods: Real-time $PM_{0.1}$, temperature, carbon dioxide (CO_2), carbon monoxide (CO), and percent relative humidity were measured at the workstation. The work process, physical characteristic of each center, and any use of controls were also recorded. Results: Occupational exposure to $PM_{0.1}$ ranged from 1,900-23,000 particles/cm³. All other IAQ parameters were within published guidelines. One center used emissions control. Exposure to $PM_{0.1}$ is significantly correlated with the number of copies produced. Conclusion: End-user risk of exposure to extremely high $PM_{0.1}$ concentrations and ENMs are common. We noted lack of controls, and existing guidelines to mitigate $PM_{0.1}$ exposures at the workstation, and a similar lack of OELs. IAQ guidelines and OELs do not adequately protect end-users from $PM_{0.1}$ and new regulatory limits are needed.

Keywords: Exposure assessment, Occupational safety, Regulatory decision making

161. Application of Bayesian Networks to Support Safety Assessment of Nanomaterials

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To perform a conventional risk assessment is not feasible for all nanomaterials (NMs) given the fast moving market of NMs. There is a need to prioritize and band/group NMs for subsequent risk assessment. In this study we explored the use of Bayesian Network (BN) modelling to predict the potential of metal and metaloxide NMs to induce a biological effect in human. BN models are very powerful because they can combine data from different nature and origins, do not need default values when data is missing and are highly flexible because new variables/ parameters and knowledge may be entered at any time as it appears to improve the model accuracy. By means of expert consultation and literature data, a BN model was constructed consisting of physicochemical characteristics of NMs (dissolution, shape, surface area, surface charge, surface coatings, surface reactivity, particle size, degree of aggregation), biological effects (neurological effects, cardio-pulmonary effects, immunological effects, inflammation, genotoxicity, reaches central nervous system, fibrosis, cell transformation) and exposure routes. The model (332 datasets) was validated with independent data (45 datasets) extracted from published studies. Based on physicochemical data, the BN model correctly predicted the potential of these NMs to induce a biological effect in 87% of the cases. The application of the BN model is shown with scenario studies for TiO_2, Ag, Co_3O_4, Cr_2O_3, CeO_2. It is demonstrated that the BN model may be used to apply safe-by-design approaches or in safety assessment to predict certain properties of a NM of which little information is available or to prioritize NMs for further screening.

Keywords: Alternative testing methods/strategies, Metal/metal oxide nanomaterials, Risk assessment

162. Production and Nutrient Composition Changes of Red Kidney Beans Exposed To Z-COTE® and Z-COTE HP1®

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The number of consumer products containing engineered nanoparticles (ENPs) has greatly increased in the last years, raising concern about their fate, transport and effects in the environment. Cosmetics and personal care (C&PC) goods have the highest number of products in the market containing ENPs. The release of these nanomaterials into the environment represents a potential pathway to reach agricultural soils and further edible plants. This research was aimed to study the impact of two regularly used ZnO ENPs (uncoated Z-COTE® and coated Z-COTE HP1®) on red kidney bean (Phaseolus vulgaris var. Red Hawk) crop plants. In this study, bean seeds were grown in natural soil embedded with Z-COTE, Z-COTE HP1, ZnO Bulk, and $ZnCl_2$ at concentrations of 0, 62.5, 125, 250 and 500 $mg \cdot kg^{-1}$ for ~100 days. The mature seeds were collected, yield was evaluated, micronutrient composition was analyzed via ICP-OES and macronutrients were quantified. Results indicated that seed production was not significantly affected. However, the number of days required to reach full maturity was impacted among treatments. Nutritional analysis revealed that Zn amounts increased in seeds exposed to 500 $mg \cdot kg^{-1}$ for all evaluated compounds, while other micronutrients remained mostly unaffected. Similarly, macronutrients did not show significant differences when compared to control seeds. The findings showed the evident Zn uptake and unnoticeable effects of ZnO exposure in bean seeds, which contribute to the work of the funding source, the University of California-Center for Environmental Implications of Nanotechnology (UC-CEIN).

Keywords: Environmental nanotoxicology, Exposure assessment, Metal/metal oxide nanomaterials, Zinc oxide nanoparticles

163. Using Earthworm Coelomocytes to Detect Toxicity of Carbon Nanotubes and Sodium Pentachlorophenol

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As standard testing organism in soil ecosystem, there have been lots of toxicity studies on Eisenia fetida. But the test at the individual level is time and animal consuming, and the sensitivity is limited. Earthworm coelomocytes were important cell in uptaking and elimination of exogenous compounds, and play an important role in the process of phagocytosis and inflammation. In this study, we explored the optimal condition to culture coelomocytes of E. fetida in vitro. And the coelomocytes were exposed to Multi-walled Carbon nanotubes (MVCNTs) and Sodium Pentachlorophenol (PCP-Na) to investigate the cytotoxicity via testing the lethal toxicity, oxidative stress, membrane damage and DNA damage. The results showed that the coelomocytes can be suspension cultured in vitro in primary under the RPMI-1640 medium with 2-4×10^4 cells/well in 96-well plate in 25 ℃, without CO_2. Both MVCNTs and PCP-Na can cause oxidative damage and produce ROS, result in lipid peroxidation with MDA generation and SOD and CAT activity inhibition at high stress. Moreover, MVCNTs and PCP-Na could damage the membrane structure of cell with the permeability of membranes increasing and the MMP inhibition. In addition, our results indicated that the toxicity of PCP-Na may be alleviated by the appearance of MWCNTs after adsorption, while PCP-Na and MWCNTs separately co-added revealed a synergetic effect based on the cytotoxicity toxicity study. In summary, coelomocyte toxicity in vitro analysis is proved to be a new sensitive method for detecting the adverse effects of various pollutants on earthworms in soils.

Keywords: In vitro toxicology, Earthworm coelomocytes, Sodium Pentachlorophenol, Multi-walled Carbon nanotubes

164. Towards the Design and Characterization of Second Generation Nanoparticles: Application of Novel Mixture Descriptors in Nano-QSPR Approaches

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Due to the extraordinary properties of surface-modified nanoparticles (so-called second generation nanoparticles, e.g. Memix@MeOx, where Memix is a bimetallic cluster of rare or noble metals, MeOx is metal oxide nanoparticle), they have a great potential for amplifying properties of unmodified nanomaterials, and extending their application and use e.g. as environmentally friendly photocatalysts. However, structural modifications and the resulting new physicochemical properties, that make the second generation nanoparticles of great industrial interest, may also create serious risk to human health and the environment. At present, there are no nanostructure-activity relationship models (nano-QSAR) for those type of systems, i.e. nanomaterials with surface modifications. Here, for the first time we implement idea of additive descriptors for mixtures, widely used for compound mixtures, to calculate physicochemical parameters of 34 samples of TiO_2 NPs modified by metallic clusters (Au, Ag, Pt), synthesized using the microemulsion method. Using this novel approach for second generation NPs, we investigated the influence of structural features of modified TiO_2 NPs on their cytotoxicity to the Chinese hamster ovary (CHO-K1) cells. Through a combination of Multiple Linear Regression (MLR) and the genetic algorithm (GA) we have developed a first ever nano-QSARmix model (R^2=0.93, Q2LOO=0.91, Q2Ext=0.88, RMSEC=0.11, RMSECV=0.13, RMSEP=0.15). This model fulfills the OECD quality recommendations and offers a meaningful mechanistic interpretation of the cytotoxicity effect of investigated second generation NPs (i.e. TiO_2 NPs surface modified with mixed metal clusters, where different amount, concentration and type of metals precursor in Memix was applied).

Keywords: Green nanomaterials, In silico modeling, Metal/metal oxide nanomaterials, Risk assessment

165. An Approach to the Characterization of Nanoparticles' Combined Toxicity: the Problem Setting and an Example of its Solving

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The more nanomaterials are used in industry, science and medicine, the more probable are multi-component impacts of these materials on humans. They arise also when nanoparticles are by-produced by long-established metallurgical and welding technologies. To start our research in combined nanotoxicology, we used suspensions of 16.7±8.2 nm NiO and/or 18.4±5.4 nm Mn_3O_4 prepared by laser ablation of 99.99% pure metals in de-ionized water. They were repeatedly injected intraperitoneally to rats at a dose of 0.50 mg or 0.25 mg 3 times a week up to 18 injections, either separately or in different combinations. Many functional indices as well as histological features of the liver, spleen, kidneys and brain were evaluated after the exposure period. Ni and Mn content of organs was measured with the help of AES and EPR methods. Both substances proved adversely bio-active, but nanoparticles of Mn_3O_4 were found to be more noxious in most of the toxicity manifestations. They induced a more marked damaging effect in the caudate nucleus and hippocampus neurons which may be considered an experimental correlate of manganese-induced Parkinsonism. Mathematical analysis based on the Response Surface Methodology revealed a diversity of combined toxicity types depending on particular effects these types are assessed for and on the effect's level. The subadditivity of unidirectional action proved to be the most frequently while the synergism - the least frequently occurring type. In this respect, there is no essential distinction between the combined toxicity of nanoparticles and that of manganese and nickel soluble salts studied by us earlier.

Keywords: Biokinetics/toxicokinetics, In vivo toxicology, Toxicological mechanisms

166. Effect of TiO_2 Nanoparticles on Bacterial Infection of HeLa Cells

Tatsiana Mironava, Yan Xu, Miriam Rafailovich. Stony Brook University, USA.

Titanium dioxide (TiO_2) is one of the most common nanoparticles found in industry ranging from food additives to energy generation. Approximately four million tons of TiO_2 particles are produced worldwide each year with approximately 3000 tons being produced in nanoparticulate form, hence exposure to these particles is almost certain.Even though TiO_2 is also used as an anti-bacterial agent in combination with UV, we have found that, in the absence of UV, exposure of HeLa cells to TiO_2 nanoparticles significantly increased their risk of bacterial invasion. HeLa cells cultured with 0.1 mg/ml rutile and anatase TiO_2 nanoparticles for 24 hrs prior to exposure to bacteria had 350% and 250% respectively more bacteria per cell. The increase was attributed to increased LDH leakage, and changes in the mechanical response of the cell membrane. On the other hand, macrophages exposed to TiO_2 particles ingested 40% fewer bacteria, further increasing the risk of infection. In combination, these two factors raise serious concerns regarding the impact of exposure to TiO_2 nanoparticles on the ability of organisms to resist bacterial infection.

Keywords: In vitro toxicology, in vitro toxicology, titanium dioxide nanoparticles, metal oxide nanomaterials

167. Effect of Cell Donor Age On Dermal Cell Response to Gold Nanoparticle Exposure

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Changes in the skin are the most prominent changes associated with aging. They include not only alteration of biochemical composition but also modification of cellular mechanics. The last one is of the particular interest considering its importance for correct physiological functioning.Here we investigate how the age-dependent changes in dermal fibroblast mechanics affect cell response to the gold nanoparticles (AuNPs). AuNPs have an enormous potential in chemical sensing, biological imaging, drug delivery, and cancer treatment. Therefore, it is important to know their potential toxicity to cells as a function of age-dependent cell mechanics.To address this issue, the AFM technique was used to examine the rigidity of fibroblasts isolated from 6 human donors of different age groups. Our results demonstrate correlation between cytotoxicity levels of AuNPs and the age of cell group. Specifically we found, that all cell groups exposed to the same concentration of AuNPs had a very similar decrease in cell proliferation. However, recovery data demonstrated that the rate of recovery from the damage is different for neonatal cells as compared to 30- and 80-years old cell group. These finding also correlates with the changes in cell membrane stiffness. Therefore, we conclude that nanoparticle uptake depends on cell membrane mechanics that in turn is a function of cell donor age.

Keywords: In vitro toxicology, metallic nanoparticles, cytotoxicity, in vitro toxicity

168. Cytotoxicity of Titanium Dioxide in Adipose Derived Stromal Cells

Tatsiana Mironava, Miriam Rafailovich, Yan Xu. Stony Brook University, USA.

The growing annual production of Titanium dioxide (TiO_2) nanoparticles lead to proportional increase in chances of occupational and consumer exposure. Considering, that these nanoparticles currently are being used in products such as pharmaceuticals, personal care, cosmetics, toothpastes, sunscreens and food additives it makes the possibility of exposure almost definite. Eventhough, in 2013 Scientific Committee on Consumer Safety stated that the use of TiO_2 nanomaterials in sunscreens, pose no adverse effects in humans when applied on healthy, intact or sunburnt skin there is still a possibility of TiO_2 penetration through compromised skin. Therefore in this study we investigate the effects of TiO_2 nanoparticles on not perfectly intact skin that due to various diseases or trauma allows nanoparticles reach living skin layer. This can be a cause of possible concern, especially when the cells exposed to particles are also exposed to bacteria commonly found on the skin surface. Specifically, we studied the interaction of Adipose Derived Stromal Cells (ADSCs) one of the cellular components of adipose tissue with two forms of TiO_2 nanoparticles, namely rutile and anatase. Our results indicate that nanosized TiO_2 adversely effects differentiation of ADSCs and have profound effects on cell function such as migration, collagen contraction, ROS and LDH formation. In addition, ADSCs pretreated with TiO_2 prior to exposure to bacteria show significantly higher rates of bacterial infections as compared to untreated control.

Keywords: In vitro toxicology, in vitro toxicology, susceptibility, metal oxide nanomaterials

169. Evaluation of Blood Coagulation to Study Protein-Nanoparticle Interactions

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Metal nanoparticles are widely used in different fields including biomedicine. There is an urgent necessity to evaluate their potential toxic effects and to relate with their physicochemical properties including their size. Among the potential hazardous effects of nanoparticles in biological systems, protein interactions can interfere with the in vivo clotting cascades resulting in serious health consequences. In the present work, we have evaluated the effect of the presence of different metal oxide nanoparticles in human plasma over blood coagulation. We have determined both thrombin time (PT) and activated partial thromboplastin time (APTT), as indicators of the extrinsic and intrinsic blood coagulation pathways. The commercial metal oxides studied were Al_2O_3 (13 nm, 50 nm and nanowires), ZnO (< 50 and < 100 nm) and TiO_2 (21 nm), and their behaviors were compared with the raw metal oxides. Results show that PT and APTT times are dependant of nanoparticle size and concentration, but with different profile depending on the nature of the chemical entity. From these observations, we can suggest that interactions among nanoparticles could account for both the formation of protein corona or agglomeration or aggregation, interfering in the coagulation process and thus further investigation is mandatory. The authors acknowledge financial support from the Ministerio de Economía y Competitividad - Spain and European Union (FEDER) (Project MAT2012-38047-C02-01)

Keywords: In vitro toxicology

170. Graphene Oxide Interactions with Innate Immune Cells: Extracellular Trap Formation and Inflammasome Activation

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The biocompatibility of graphene oxide (GO), especially its interactions with immune system, should be controlled for successful biomedical applications. The innate immune system rapidly detects invading foreign intruders and eliminates them, and neutrophils act as a first line of defense. Neutrophils can engulf and digest microbes or can release neutrophil extracellular traps (NETs) to digest microbes extracellularly. Here, we asked whether neutrophils also are capable of 'sensing' GO. Interaction of freshly isolated primary human neutrophils with endotoxin-free GO (small and large flakes) produced by modified Hummers method was studied. Cell viability was determined by measuring intracellular ATP. Using TEM, we noted that GO flakes were aligned with the cell membrane leading to membrane stripping, and this effect was more pronounced for the large flakes. Furthermore, GO triggered NET formation in a size-dependent manner as evidenced by SEM and confocal microscopy. Size-dependent GO degradation by myeloperoxidase (MPO) present in NETs and in degranulating neutrophils was observed. We also evaluated interaction of GO with primary human monocyte-derived macrophages and found that small and large GO flakes were readily internalized. No cytotoxicity was detected. Then, multi-plex arrays were employed for screening of inflammatory responses in LPS-primed or unprimed cells. GO induced caspase-dependent IL-1β expression, a hallmark of inflammasome activation, in LPS-primed macrophages. Overall, our work has provided new insights regarding the interaction of GO with macrophages and neutrophils and revealed cell type-specific differences.

Keywords: Carbon-based nanomaterials, Human toxicology, Toxicological mechanisms, Mechanism of Inflammasome activation, graphene biodegradation

171. Silver Nanoparticles and Sinorhizobium meliloti Nodulation on Alfalfa Roots

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Background: Sinorhizobia are a useful group of soil bacteria which induce nitrogen-fixing nodules on leguminous plants. Sinorhizobium (Ensifer) meliloti coexists with alfalfa roots to fix atmospheric nitrogen. The purpose of this study was to evaluate the effect of silver nanoparthcles (SNPs) on nodulation of S. meliloti in alfalfa roots. Methods: Bacteria were isolated from nodules of alfalfa plants collected from several areas of Khorasan- Razavi province in Iran. Isolates were identified by different biochemical tests and confirmed by nodulation test and colony- PCR. The effect of SNPs was evaluated on the growth of bacteria in YEMB (Yeast extract mannitol broth) and YEMA. The effect of different concentrations of SNPs (250- 3000 ppm) was examined on the nodulation of alfalfa (variety Hamadani) roots in Finish Peat media (without nitrogen). Formation of nodules in the treated plants was compared with the control plants. Results: nif gene was detected in all 15 isolates by colony PCR. The growth of isolates was significantly reduced by increasing concentrations of SNPs in liquid and solid media. By increasing concentrations of SNPs, the size and number of nodes and length of plants significantly decreased in alfalfa plants and at higher concentrations nodes were not formed. The results showed that SNPs can decrease the growth of S. meliloti and also nodulation in alfalfa plants. Inappropriate entering of SNPs to soil may have a negative impact on the number of S. meliloti bacteria and may decrease the number of these beneficial bacteria in the soil gradually.

Keywords: Environmental nanotoxicology, Sinorhizobium meliloti, Alfalfa, Biological nitrogen fixation, Silver nanoparticles.

172. Quercetin and Melatonin in Ameliorating Titanium Dioxide Nanoparticles Neurotoxicity Effects in Wistar Rats: Role of Mitochondria

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The study aims at unveiling the fate of titanium dioxide nanoparticles (TNPs) when administered intravenously to adult male Wistar rats and their attenuation by natural compounds. Toxicological effects of TNPs have raised the concern over the human health and the environment though; they have been widely used in medicine, cosmetics and other conventional uses because of their greater effectiveness, specificity and high refractive index. In our study, the adult male rats were treated with melatonin (5 mg/kg, orally) and quercetin (5mg/kg, orally) for 14 days and TNPs (5mg/kg, intravenously) for 7 days consecutively. The study was conducted in mitochondria isolated from brain of rats. Oxidative stress indexes including enzymatic and non enzymatic antioxidants indicate that TNPs may pose adverse health risk to mitochondrial brain with the generation of reactive oxygen species and lead to neuronal cell death, therefore the proper usage of TNPs in the environment is the need of hour. This study investigates possible pathways by which TNPs when administered could cross the (Blood brain barrier BBB) by employing both toxicity and mechanistic studies and also attenuate the effect of TNPs in combination with Quercetin and Melatonin in different regions of brain of rats under in vivo conditions. Source of funding: Council of Scientific and Industrial Research (CSIR).

Keywords: Systems biology/toxicology, Titanium dioxide nanoparticle, neurotoxicity, intravenous, Blood Brain Barrier, quercetin, Melatonin

173. Green Synthesis of Phytochemical-Stabilized Nontoxic Silver Nanoparticles and Their Chemopreventive Potential

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Green chemistry methods offer opportunities to design greener production technologies with safer nanomaterial, reduced environmental impact, waste reduction and energy efficiency. In this study an attempt has been made for phytofabrication of silver nanoparticles by reducing silver with aqueous bark extract of Saraca asoca (Roxb.) (belonging to family Caesalpiniaceae) having high concentration of flavonoids, terpenoids, saponins, phenol, tannin, lignin, cardiac glycosides and proteins. The properties of these green-Au NPs were characterized by TEM, UV/Vis and FT-IR spectroscopy and the Au NPs exhibited excellent homogeneity with an average diameter of 3-10 nm and high dispersity at all pH ranges, with long-term stability as well as excellent cytocompatibility. FT-IR analysis of surface property of fabricated nanomaterial and GC-MS analysis of extracted phytochemical stabilizers indicated the involvement of carboxyl (-C=O), hydroxyl (-OH) and amine (-NH) functional groups of the phytochemicals present in S.asoca bark extract in reducing, capping and stabilizing silver nanoparticles. The potential toxicological effect of these particles has been studied using in vitro cytotoxicity analysis as well as in vivo mouse model. No significant level of toxicity was observed at highest dose of 5000 mg/Kg body weight. The chemopreventive properties of the phytofabricated nanomaterial was significant as analysed using benzene exposed secondary acute myelolytic leukemia mouse model. This novel synthesis route for Au NPs using plant extract reducing agent may be effectively exploited for nontoxic energy efficient nanomaterial for medical applications.

Keywords: Biomedical/therapeutic applications, Green nanomaterials, In vivo toxicology, Green material, Chemoprevention

174. Nano Inside and Outside– Informing the Public on Safety Aspects of Nanomaterials- From Experts to Laypersons

Katja Nau[1], Nils Bohmer[4], Harald Krug[4], Dana Kuehnel[2], Clarissa Marquardt[1], Florian Paul[3], Christoph Steinbach[3]. [1]Karlsruhe Institute of Technology, Germany; [2]Helmholtz Centre for Environmental Research; [3]Society for Chemical Engineering and Biotechnology (DECHEMA); [4]Swiss Federal Laboratories for Materials Science and Technology, Switzerland.

The success of nanotechnology is particularly based on its versatility having a great impact on our daily life ranging from electronics to the health care system. However, consumers and students often miss reliable and understandable information on nanotechnology, especially on nanomaterials and their applications, and don't know where to get such information. There is a high demand for answers to questions such as "What is a nanoparticle?" or "Are there any risks for myself or the environment?". Equally, communicating scientific facts with the public is an ambitious task in general as complex issues need to be simplified whilst ensuring scientific correctness at the same time. Due to the multidisciplinary nature of nanotechnology, the communication on the related safety aspects is particularly challenging. But as many products of everyday life contain nanomaterials, there is a high public interest in reliable, quality approved and straightforward information. Hence, the DaNa2.0 project is addressing these challenges by collecting and evaluating scientific results in an interdisciplinary approach with scientists from different research areas. Evaluated research findings from the field of human and environmental nanotoxicology are presented in a worldwide unique kind, correlating material properties and possible applications. Information depth is tailored to interested laypersons, students and stakeholders on the website "www.nanoobjects.info". This platform offers reliable data and information on the 26 most widespread used nanomaterials together with answers to frequent questions as well as various cross-cutting topics like toner or nanomedicine. DaNa2.0 is funded by the German Federal Ministry of Education and Research (FKZ 03X0131).

Keywords: Environmental nanotoxicology, Human toxicology, Nanosafety

175. Splenic Capture and Degradation of Graphene Oxide In Vivo

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Graphene nanomaterials are fuelling a revolution in material science and technology, ranging from aerospace engineering to electronics. Among those, various applications in biomedicine, such as biosensors and drug delivery systems are being developed using various types of graphene-based materials, predominantly graphene oxide (GO). For this reason, it is imperative to determine the safety and toxicological profile from exposure to GO. We recently reported that single or few layer graphene oxide flakes are predominantly excreted in the urine, and the fraction of material that remains in the body is captured by the spleen over a 24h period following intravenous administration. Here, we determined the exact localisation, long-term fate and biodegradability of GO in the spleen over a 9 month period following a single intravenous administration. Using a combination of immuno-staining, cell sorting and confocal Raman mapping, we found that GO was internalised preferentially within a specialised sub-population of splenic macrophages, the marginal zone macrophages. Using TEM coupled with electron diffraction, we confirmed the sub-cellular internalisation of GO flakes into vesicular structures of spleen macrophages. The gradual change of the graphene oxide structure in situ was carefully followed by Raman spectroscopy and TEM coupled with electron diffraction, ultimately providing unambiguous evidence of the degradation of GO. Our findings bare important implications for the long term toxicity and safety profile of GO materials following administration and exposure of living tissues specifically in the context of clinical applications. Source of Funding: UK Engineering and Physical Sciences Research Council and EU-FP7-Graphene Flagship (Grant #604391).

Keywords: Carbon-based nanomaterials, Emerging nanomaterials

176. Safety Assessment of Four Iron-Based Nanomaterials Developed for In Situ Remediation of Groundwater Pollutants Using Green Alga *Chlamydomonas* sp.

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Four iron-based nanomaterials were modified: FerMEG12 (Milled-Fe) by UVR-FIA GmbH, Carbo-Iron (Carbo-Fe) and Fe-TZB223L (Fe-zeolite) by GmbH-UFZ , and BioremFX (Fe-oxide) by HMGU, largely used for clean-up of groundwater pollutants, mainly halogenated organics and toxic metal ions. Hence, careful assessment of their possible harmful potential prior to their release into the environment is required. The unicellular green alga Chlamydomonas sp., a primary producer in a healthy ecosystem, was chosen as a microorganism model to test the potential effect of these iron-based nanoparticles. Algal growth, chlorophyll fluorescence, photosynthetic efficiency, membrane integrity and reactive oxygen species (ROS) generation were examined after Chlamydomonas sp. exposure to 10, 50 and 500 mg/l of the nanomaterials. Fe-NMs sizes and charges in the exposed medium were also examined. Algal cells were more affected after short-term exposure (2h) than after longer time exposure (24h) to all tested Fe-NMs. Survived algae partly produced new generation after 24h, and chlorophyll fluorescence together with photosynthesis activity was recovered. Consistently with these results, ROS was significantly generated after 4h and decreased again after 24h. The Z average size and zeta-potential of all Fe-NMs in algal medium did not change during toxicity tests. Among the tested materials, Milled-Fe was found to have the strongest negative effect, while Fe-oxide was the safest material. It is recommended that Fe-NMs should be performed on different microorganisms and longer term exposure to have a broad view of safety materials.

Keywords: Emerging nanomaterials, Environmental nanotoxicology, Exposure assessment

177. Time-Dependent Up-Regulation of Biotransformation and Oxidative Stress Genes Following Sub-Acute Inhalation of Combustion-Derived Ultrafine Particles in Mice

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There has been considerable research on the potential toxic effects of ultrafine particles (UFP) (<100 nm) on human health. Long-term health consequences, however, including altered gene expression, caused by exposures to combustion-derived UFP have received insufficient attention. Butadiene soot, generated from the incomplete combustion of 1,3-butadiene (BD), is both a model UFP mixture and a real-life example of a petrochemical product of incomplete combustion. In the present study, we investigated the extent of mouse lung recovery 10 days after inhalation exposures to BD UFP had ended. Female BALB/c mice exposed to either filtered air or to BDS (5 mg/m^3, 4 hour/day, 21 days) were sacrificed immediately, or 10 days after the final BDS exposure. Bronchoalveolar lavage fluid (BALF) was collected for cytology and cytokine analysis. Lung proteins and RNA were extracted for protein and gene expression analyses, and lung histopathology was performed. Sub-acute exposures of mice to hydrocarbon-rich UFP induced: BALF neutrophil elevation; lung mucosal inflammation, and increased BALF IL-1β concentration. All three outcomes returned to baseline levels 10 days post-exposure. In contrast, lung connective tissue inflammation persisted 10 days post-exposure. We detected time-dependent up-regulation of biotransformation and oxidative stress genes, with incomplete return to baseline levels; and observed persistent particle load in alveoli following 10 days of recovery. In summary, ten days after a 21-day exposure to BD UFP has ended, incomplete lung recovery contributes to a pro-biotransformation, pro-oxidant, and pro-inflammatory milieu, which may be a starting point for potential long-term cardio-pulmonary effects.

Keywords: Environmental nanotoxicology, Genotoxicity, In vivo toxicology

178. Genotoxicity of Metal Nanoparticles – Possible Nanospecific Effects

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Nanosized metal particles can roughly be divided in poorly soluble ("inert") and partly soluble materials. Inert particles not possessing intrinsic genotoxicity may be genotoxic via indirect generation of reactive oxygen species, as driven by particle characteristics. The toxicity of partly soluble particles additionally depends on the influence of the soluble form. A key question is, whether nanosized particles have specific or higher genotoxic effects in comparison with similar particles of larger size. We have assessed the genotoxicity of different metal based particles, TiO_2 representing inert particles and ZnO and CuO partly soluble particles in human bronchial epithelial BEAS-2B cells, using the alkaline comet assay and the micronucleus assay for DNA and chromosome damage, respectively. Particle size distribution in dispersions was examined by dynamic light scatter and cellular uptake of the particles by hyperspectral microscopy and electron microscopy. As expected, partly soluble particles were more cytotoxic and genotoxic than inert particles. CuO nanoparticles showed a higher cellular uptake and genotoxic effect than fine CuO particles. Size-specific differences in uptake and toxic effects were also observed between nanosized and fine TiO_2. The genotoxic effects of ZnO appeared to occur primarily at a narrow cytotoxic dose-range. Nanospecific genotoxic effects of small metal-based particles may derive from size-dependent cellular uptake of particles and (for partly soluble particles) intracellular dissolution. Particle presence in cells appears to result in persistent or continuous DNA damage, which may, in addition to secondary genotoxicity, have significance in particle carcinogenesis. [Supported by Finnish Work Environment Fund (No. 112248)]

Keywords: Carcinogenicity, Genotoxicity, In vitro toxicology, Metal/metal oxide nanomaterials, Copper oxide, Titanium dioxide, Zinc oxide

179. Prenatal Exposure to Carbon Black Nanoparticle Induces Protein Conformational Change in the Brain Determined by In Situ Fourier Transform Infrared Spectroscopy (FT-IR)

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Central nervous system is one of the major targets of the developmental toxicity of nanoparticles. Our previous report indicated that prenatal exposure to carbon black nanoparticle (CB-NP) induced chronic and diffuse perivascular abnormalities in the brain of offspring (Onoda et al., 2014). The aim of the present study was to characterize of the perivascular abnormalities using in situ fourier transform infrared microspectroscopy (in situ FT-IR). Pregnant ICR mice were treated with CB-NP suspension (95 μg/kg/time) by intranasal instillation on gestational days 5 and 9. Cerebral cortex were collected at 6 weeks after birth. Reflective spectra of in situ FT-IR were acquired by lattice measurement (X-axis: 7, Y-axis: 7, 30 μm apertures) centered around a blood vessel with perivascular abnormalities, and mapping analysis of protein secondary structure were performed. In situ FT-IR revealed an increase in β-sheet component and a decrease in α-helix component in the brain of CB-NP exposure group. The observations were remarkable around the vessel with perivascular abnormalities where enlarged lysosome granules in perivascular macrophages and astrocytes with GFAP high-expression were confirmed. These data indicated that β-sheet-rich protein was accumulated on the region of the perivascular abnormalities by maternal exposure to CB-NP. Some proteins may be denatured and discharged to perivascular regions, which is the clearance route of brain waste, by maternal exposure to nanoparticle. The protein conformational change may be key factor to elucidate the underlying mechanism of the developmental toxicity of nanoparticles in the brain. Funding: This work was supported by Grant-in-Aid for JSPS Fellows (15J05718).

Keywords: Developmental nanotoxicology, in vivo toxicology, Brain, Blood vessel, Protein conformation, Astrocyte, Perivascular macrophage

180. Unique Cellular Responses to Distinct Nanoparticle Properties Uncovered by Single Cell RNA-Seq

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One challenge in establishing clear relationships between distinct nanoparticle (NP) properties and cellular response originates from the experimental difficulty to achieve uniform NP loads in the studies cells. The uneven load creates heterogeneous cell populations with some cells “overloaded” while other cells are loaded with few or no NPs. Yet gene expression studies have been conducted exclusively in the population as a whole, identifying generic responses, while missing unique responses due to signal averaging across many cells, each carrying different loads. Here we applied single-cell RNA-Seq to alveolar epithelial cells carrying defined loads of aminated or carboxylated quantum dots (QDs), showing higher or lower toxicity, respectively. Interestingly, cells carrying lower loads responded with multiple strategies, mostly with upregulated processes, which were nonetheless coherent and unique to each QD type. In contrast, cells carrying higher loads responded more uniformly, with mostly downregulated processes that were shared across QD types. Strategies unique to aminated QDs showed strong upregulation of stress responses, coupled in some cases with regulation of cell cycle, protein synthesis and organelle activities. In contrast, strategies unique to carboxylated QDs showed upregulation of DNA repair and RNA activities, and decreased regulation of cell division, coupled in some cases with upregulation of stress responses and ATP related functions. Together, our studies suggest scenarios where higher NP loads lock cells into uniform responses, mostly shutdown of cellular processes, whereas lower loads allow for unique responses to each NP type that are more diversified, proactive defenses or repairs of the NP insults.

Keywords: Adverse outcomes pathway analysis, Biomedical/therapeutic applications, In vitro toxicology, Systems biology/toxicology, Toxicological mechanisms

181. Organ-Specific and Size-Dependent Ag Nanoparticle Toxicity in Gills and Intestines of Adult Zebrafish

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As a result of their attractive antimicrobial properties, silver nanoparticles (AgNPs) are one of the most abundant and commercially available with over 400 nanoproducts containing AgNPs.AgNPs are found in many consumer products such as cosmetics and textiles. Consequently, this amplifies the likelihood of AgNPs reaching water systems with the possibility of exposing aquatic organisms that reside there. It is therefore imperative to know and understand the hazard assessment of these AgNPs to aquatic life forms such as fish through a comprehensive comparative analysis. We carried out an in vivo study on adult zebrafish to determine if the size of 20 nm and 110 nm citrate-coated AgNPs differentially impact the gills and intestines. Ionic $AgNO_3$ served as a positive control. Following exposure for 4 hours, 4 days or 4 days plus a 7 day depuration period, we obtained striking size dependent differences via toxicokinetic profiles (both particle types were retained in the intestines until after depuration confirming bioaccumulation), histopathology and Ag site deposition in the target organs (n.b. 20nm found in basolateral membrane). We linked the deleterious consequences to a disruption of the Na+/K+ ion channel in both target organs. We demonstrate mechanistically size dependent differences (20 nm particles caused significantly higher inhibition) confirmed by a reduction in ATPase activity and immunohistochemical detection of the α subunit of this channel. These results demonstrate the importance of particle size in determining the hazardous impact of AgNPs in the gills and intestines of fish in the environment. The research was supported by the National Science Foundation and the Environmental Protection Agency.

Keywords: Biokinetics/toxicokinetics, Environmental nanotoxicology, In vivo toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

182. Relationship Between Physicochemical Characteristics of Engineered Nanomaterials and Cytotoxicity Assessed by Impedance-Based Monitoring

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Introduction: Engineered nanomaterials (eNMs) have unique desirable properties, but can also induce toxic effects. The central aim of this project was to assess key physicochemical properties of eNMs that are relevant for acute toxicity. Methods: The NANOGENOTOX protocol was used to produce dispersions of reproducible quality of seven eNMs: NM-302 and NM-300k (Ag: 200 nm rods and 16.7 nm spheres, respectively), NM-200 and NM-203 (SiO_2 spherical amorphous: 18.3 nm and 24.7 nm, respectively), and NM-100, NM-101 and NM-103 (TiO_2: 100 nm anatase, 6 nm anatase, and 24.7 nm rutile, respectively). Characterization in batch solutions and exposure media was carried out by dynamic light scattering (DLS) and transmission electron microscopy. High throughput toxicity assessment was performed using a real-time non-invasive impedance-based method to assess cell viability and proliferation. Two cell types, the A549 adenocarcinomic human alveolar basal epithelial cell line and primary gingival fibroblasts, were exposed to eNMs at clinically relevant concentrations (2, 10, 20, 50, and 100 µg/mL) for 24 hrs, and monitored during the entire period of exposure. Internalization was visualized by ultra-high resolution dark-field microscopy. Conclusions: DLS results revealed a good dispersion reproducibility. The toxicity ranking was: NM-300k > NM-302 > NM-101 > NM-203 > NM-200 > NM-103 > NM-100. This indicates that solubility, size and shape play a role in acute cytotoxicity. This study was part of the FP7 EU “NANoREG” and NANO2021 “NorNANoREG” projects. ENMs were provided by the JRC Nanomaterials Repository.

Keywords: High throughput screening, In vitro toxicology, Physicochemical characterization

183. Quantum Dots Nanoparticles Induce Apoptosis in Chinese Hamster Fibroblast Cell Lines (V79)

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In recent years, due to the increases in the technologies and the uses of the nanomaterials, nanotoxicology has been the subject of many researches.Despite the potential advantages of the nanoparticles, it has been suggested to trigger undesirable hazardous interactions with biological systems to generate harmful effects.Therefore, there is an increasing concern about the potential effects on human health and environmental effects. In this study, it was aimed to evaluate the genotoxic effects of quantum dots (QDs) which have been aimed to be used in many areas such as bioimaging, diagnosis and mechanics. The apoptotic changes induced by 2 mercaptopropionic acid (2-MPA)-coated silver sulfide QD was evaluated by RT-PCR technique in V79 cells.There are several genes known to involve in apoptotic pathways.The RT-PCR results showed that 2-MPA QD at the concentrations 10-500 μM affected the apoptotic genes (bax, bcl2 and caspase-9) in V79 cells.More detailed studied should be performed to clarify the apoptotic mechanisms of QDs. Acknowledgement: This study was supported by TUBITAK (Project Number: 114S861).

Keywords: In vitro toxicology, Toxicological mechanisms, quantum dots, nanotoxicology, V79 cells, 2-mercaptopropionic acid (2-MPA)-coated silver sulfide QD

184. The Effect of Topically Applied Carbon Nanomaterial on Skin Inflammation in a Model of Allergic Contact Dermatitis

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Consumer and industrial applications of carbon nanomaterials (CNM) are expanding, which has increased the potential for human skin exposure and the need for CNM toxicological evaluation. Carbon nanotubes (CNTs) are currently being used or developed for biomedical imaging systems, transdermal drug delivery, textiles, and sport equipment. Early work on CNT dermal toxicity examined the effects of high dose CNTs; however, additional research is needed to identify the effects of more biologically relevant, low dose CNM exposure and its impact on development of skin inflammatory diseases. Allergic contact dermatitis (ACD) is characterized by skin swelling and inflammation upon contact with a chemical hapten or allergen. ACD has a prevalence of 15 - 20% in the general population, but the prevalence is highly dependent on occupation, since workplace allergen exposures are common in industrial settings. Co-exposure of allergens and CNTs is likely highest for industrial occupations, but it is expected to rise when more CNT based consumer products become a reality. We hypothesize that carbon nanomaterial exposure to skin will be immunomodulatory and alter the progression of allergic contact dermatitis. Preliminary data generated in our lab has shown a significant increase in mast cell degranulation and skin swelling when carboxylated multi-walled CNTs and dinitrofluorobenzene (DNFB) are administered together in a low dose, 1 µg bolus during the challenge phase of an ACD model. Identification of the possible hazards involved with CNM dermal application may help in exposure risk assessment and the design of safer materials. Funding Sources: NIEHS Training Grant ES07026, NIH RO1 ES021492

Keywords: Carbon-based nanomaterials, In vivo toxicology, Occupational safety

185. Genotoxic Effect of Copper Oxide Nanomaterial on Hepatocellular Carcinoma (C3A) Cell Line

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Copper nanomaterials (NMs) are widely used in different company sectors for both the high antimicrobial properties and the low toxic activity against mammals. This study, performed within the frame of FP7-SUN (Sustainable Nanotechnologies) project, aimed to evaluate the effects of copper oxide nanomaterials (CuO NMs) in vitro using hepatocellular carcinoma cell line (C3A) and the genotoxic effect in terms of single/double DNA strand breaks and oxidative damage using the fpg-modified alkaline comet assay. Two different sub lethal doses identified during cytotoxicity tests using benchmark dose approach were analysed with the addition of $CuSO_4$ salt as control; finally, a further study to check possible interference was performed. After four hours exposure, CuO NMs shows a significant genotoxic effect on C3A in both the sub lethal doses (25.8 and 12.9 µg/ml) compared to control compound ($CuSO_4$); most of the genotoxic effect is more likely linked to a direct breakage of the DNA strand rather than an oxidative damage. CuO NMs does not show significant interference in this assay.

Keywords: Genotoxicity, Human toxicology, In vitro toxicology, Metal/metal oxide nanomaterials

186. Zebrafish Embryo Toxicity Assessment and Bioaccumulation of Carbon Nanotubes Suspended in Pluronic® F-108

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Background and rationale: Carbon nanotubes (CNTs) are often suspended in poloxamer surfactants by sonication, which may confound toxicity studies because sonication of poloxamers, such as Pluronic® polymers and polyethylene glycol (PEG), can create degradation products that are toxic to mammalian cells (Wang et al., Nanotoxicology, 2013, 7, 1272; Murali et al., Exp. Biol. Med., 2015, 240, 1147). To investigate whether sonolytic products were also toxic to organisms, herein we present a toxicity assessment of Pluronic® F-108 with and without suspended CNTs using embryonic zebrafish as an in vivo model. Results: Pluronic® sonolytic degradation products were toxic to zebrafish embryos just as they were to mammalian cells. When the toxic Pluronic® fragments were removed, there was little effect of pristine multi-walled CNTs (pMWNTs), carboxylated MWNTs (cMWNTs), or pristine single-walled carbon nanotubes (pSWNTs) on embryo viability and development, even at high CNT doses. To quantify CNTs associated with organisms, a gel electrophoretic method coupled with Raman imaging was developed to measure the bioaccumulation of CNTs by zebrafish embryos, and a dose-dependent uptake of CNTs was observed. Impact: These data indicate that embryos accumulate pMWNTs, cMWNTs, and pSWNTs yet there is very little embryo toxicity (Wang et al., Nanotoxicology, 2016, in press). This work was funded by the Semiconductor Research Corporation Engineering Research Center for Environmentally Benign Semiconductor Manufacturing (Grant ERC 425.048) and the National Institute for Environmental Health Sciences (Grant R15-ES023666) awarded to UTD; and, the National Institute of Health (Grants ES017552-01A2, ES016896-01, P30 ES000210, and AFRL FA8650-050105041) awarded to OSU.

Keywords: Alternative testing methods/strategies, Carbon-based nanomaterials, Environmental nanotoxicology

187. Hazard Evaluationin Guidenano: A Web-Based Guidance Tool for Risk Assessment and Mitigation of Nano-Enabled Products

Margriet Park[1], Gemma Janer[2], Joan Cabellos[2], Julia Catalan[3], Maria Diez[2], Maria Luisa Fernandez-Cruz[4], Natalia Ferraz[5], David Hernandez-Moreno[4], Juan Jose Izquierdo-Galvez[4], Viviana Lopes[5], Marianna Matzke[6], Helene Stockmann-Juvala[3], Socorro Vázquez-Campos[2], Claus Svendsen[6]. [1]RIVM, Netherlands; [2]Leitat, [4] INIA, Spain; [3]FIOH, Finland; [5] Uppsala University, Sweden; [6]NERC, UK.

Within the EU FP7 funded project GUIDEnano a web-based guidance tool is developed which enables the user to evaluate and manage human and environmental health risks of nano-enabled products considering the whole product life cycle: synthesis of nanomaterials (NM), manufacturing of NM-enabled products, use, and end-of-life phase (including recycling). The tool's quantitative human and environmental hazard evaluation is done based on information incorporated in the tool or available to the user. This information is organized in the following way: Previously derived hazard threshold values (e.g. derived no effect level-DNEL, predicted no effect concentration-PNEC) for the exposure-relevant NM. If hazard threshold values are available for similar materials, the level of similarity will be evaluated and taken into account. Data from individual toxicity studies with the exposure relevant NM or similar. The tool facilitates evaluation of each study using criteria related to similarity between the exposure-relevant NM and the tested material, quality of the data, relevance of the study for each given endpoint, making use of all available information, including studies that do not comply with conventional test guidelines. Where no hazard value and insufficient relevant test information is available, the tool will offer the use of conservative default hazard values for general NM categories The use of information from non-conventional hazard studies as well as information on NMs which are slightly different from the exposure relevant materials introduces a certain level of uncertainty. The sources of this uncertainty will be made explicit and taken into account in the overall risk assessment.

Keywords: Alternative testing methods/strategies, Life cycle analysis, Risk assessment

188. Effects of Particle Surface Charge on Inhaled Aerosolized Mesoporous Silica Nanoparticles

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Inhalation is one method of therapeutic drug delivery that is both effective and simple for patients. Depending on drug particle size, the inhaled drug can deposit in the deep lung either for localized treatment or for transport into the central nervous system. Mesoporous silica nanoparticles (MSN) have shown great potential in drug delivery due to their architecture, functionality, and biocompatibility. We wish to investigate the influence of nanoparticle charge and its effects on deposition, uptake and translocation in the respiratory tract. Two sets of fluorescently-labeled 50 nm mesoporous silica nanoparticles, one with a positive surface charge and one with a negative surface charge, were aerosolized into aqueous droplets to model particulate matter. CD-1 male mice were exposed to either the positive or negative MSN aerosol for 5.5 hours. The mice were sacrificed immediately, 1 day, 7 days, or 21 days after the exposure. These timepoints allow for both acute and long-term effects of exposure to be analyzed. BALF (bronchoalveolar lavage fluid) will be analyzed for inflammatory responses and fluorescent bservation of nanoparticle uptake. TEM will also be used to visualize the effects of charged particle uptake by macrophages in the BALF. Our most current results from this work will be presented. This study is funded by the Integrated Health Sciences Facility Core at UC Davis.

Keywords: Biomedical/therapeutic applications. In vivo toxicology, Drug delivery

189. Investigation of Acute Toxicity of Perovskite Nanomaterials (A New Solar Cell Nanomaterial) in Zebrafish

Danae Patsiou, Ross Alexander, Theodore B. Henry, Teresa F. Fernandes. Heriot-Watt University, UK.

Next generation solar cells incorporate perovskite-based nanomaterials that demonstrate high stability and efficient capture of solar energy for the generation of electricity. While the attributes of perovskite nanomaterials are being realised, any potential consequences of the technology, including their toxicology upon release into the environment, must be assessed. Perovskite materials have the ABX3 crystal structure and the standard compound is methylammonium lead triiodide ($CH_3NH_3PbI_3$). The chemical reactions of perovskite materials in the aqueous phase can lead to release of Pb^{2+} and consequent toxicity in aquatic organisms. Our objective was to assess acute toxicity of two perovskite nanomaterials ($CH_3NH_3PbI_3$ and $CH_3NHNH_3PbBr_3$) in zebrafish larvae. The average diameter of perovskite $CH_3NH_3PbI_3$ particulates in medium was 141.6±30.85 nm measured by Dynamic Light Scattering. The median lethal concentration (LC_{50}) was greater than 200 mg/L in zebrafish larvae exposed for 96 hours (from age 72 to 168 hours post fertilization). Parallel experiments with Pb^{2+} suggest that much of the toxicity may be attributed to the presence of Pb^{2+} in the perovskite nanomaterials. Further experimentation will investigate the relation between perovskite nanomaterials and Pb-related toxicity endpoints, and consider effects of dietary exposure to these nanomaterials in adult zebrafish. Parallel work is being conducted on the model microalgal species Chlorella vulgaris. Results from this work will inform the safe development of nanotechnology applied to solar cell systems.

Keywords: Emerging nanomaterials, Perovskites, zebrafish, solar cells nanotechnology

190. Anthracene Sorption to TiO_2 Nanoparticles and Bioavailability of UV-Activated Anthracene By-Products in Larval Zebrafish

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Engineered nanoparticles (NPs) can be released into aquatic environments and present a risk of toxicity for aquatic organisms. Among the most commonly used NPs are TiO_2-NPs, and, although these NPs appear to be of minimal toxicity, their potential for photo-activity and sorption to toxic substances are persisting environmental concerns. Several studies have shown sorption of organic compounds to TiO_2-NPs and enhanced toxicity of the co-contaminants. The toxicity of polycyclic aromatic hydrocarbons (PAHs) has been extensively studied and there is information on the toxicity of UV-activated PAHs but the interaction between TiO_2-NPs, PAH sorption, and UV-activation is unknown. The objective of this study was to evaluate sorption of anthracene to TiO_2-NPs by 1) analysis of the preparations with and without UVA irradiance by fluorescence spectroscopy and 2) assessment of changes in expression of target biomarker genes including cytochrome P450 1A (cyp1A), DNA repair (ddb2), superoxide dismutase (sod1) and aryl hydrocarbon receptor 2 (ahr2) genes, in larval zebrafish. Zebrafish larvae (72 hpf) were exposed (24 h) to anthracene (0-30 µg/L) in freshwater ($[Ca^{2+}]$ = 71 mg/L according to OECD no. 203). Preparations were exposed to 80 kJ/m^2 UVA at the end of the 24 h exposure, and larvae were sampled at 3 h after UVA exposure. For experiments in which sorption of anthracene to TiO_2-NPs (4-8 nm diameter) was investigated, TiO_2-NPs (2 mg/L) were added to anthracene preparations and stirred for 24 h prior start of exposure. Anthracene was not acutely toxic to zebrafish larvae at the concentrations tested. After the UVA exposure, zebrafish mortality was 28±8% (±SD, n=3) at the highest anthracene concentration (30 µg/L). When fish were exposed to anthracene and UVA, a 45-fold increase in cyp1A expression and a 1.7 fold-induction of sod1 were observed at 15 µg/L anthracene. The presence of 2 mg/L TiO_2-NPs with anthracene resulted in no significant change in expression of either cyp1A or sod1 after UVA exposure compared to unexposed controls. For fish exposed to TiO_2 (2 mg/L) and UVA without anthracene there was no induction of either cyp1A or sod1, which is in accordance with other studies that have also not observed photo-induced toxicity at relatively low TiO_2-NPs concentrations. Future work will investigate the association between TiO_2-NPs and PAHs and the products of photo-induction.

Keywords: Environmental nanotoxicology, PAHs, titanium, nanoparticles, zebrafish

191. Nanotoxicological Effects of Nanostructured Hydroxyapatite for Pseudokirchneriella Subcapitata Microalgae

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Hydroxyapatite (HA) nanoparticles ($Ca_{10}(PO_4)_6(OH)_2$) can act as nanostructured fertilizers, which is a promising alternative for replacing conventional fertilizers with numerous advantages. However, the use of the nanomaterials as fertilizer also raise concerns about side effects caused by the accidental release in the environment, which can impact aquatic biota and consequently the human health. The present study aims to synthesize and characterize the physico-chemical properties of hydroxyapatite nanoparticles, and to evaluate its nanotoxicity for aquatic microorganisms, such as Pseudokirchneriella subcapitata algae, since microalgae form the base of the aquatic trophic chain. The synthesis of HA nanoparticles was carried out by coprecipitation method using as reagents calcium nitrate ($Ca(NO_3)_2$) and ammonium phosphate $(NH_4)_2HPO_4$ followed by hydrothermalization at 150°C for 120 minutes, which is simple, cheap and fast reaction pathway. Subsequently, the nanoparticles were characterized by scanning electron microscopy (SEM), X ray diffraction (XRD), zeta potential and isotherms adsorption/desorption of N_2 for structural and morphological characterization. Following the standard methodology proposed by OECD, 2011, we performed toxicity tests for microalgae to evaluate the inhibition of growth when exposed to the HA nanofertilizer. The results showed that the algal growth was significantly inhibited for high concentrations of Coprecipitated HA samples, when compared with the control sample and Hydrothermalized HA. In addition, changes in particle size, surface area, surface charge stability and formation of agglomerates/aggregates of the nanoparticles influences algae toxicity and are currently under investigation.

Chinnamuthu, C.R.; Murugesa Boopathi, P. Nanotechnology and Agroecosystem. Madras Agric. J., Coimbatore, v. 96 (1-6), p. 17-31, 2009.

Keywords: Environmental nanotoxicology, Environmental nanotoxicology; nanofertilizer; hydroxyapatite; microalgae

192. Comparative Lung Bioactivity of Vapor Grown Carbon Nanofibers and Multi-Walled Carbon Nanotubes

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Vapor grown carbon nanofibers (VGCF®-H) and multi-walled carbon nanotubes (MWCNT-7) are examples of two-dimensional carbon based nanoparticles. Our laboratory has previously investigated the bioactivity of MWCNT-7, and in the present study, VGCF®-H, in the lung. This puts our laboratory in a unique position to compare the biological responses and mechanisms of these nanoparticles. In the present study, male C57 mice were exposed to VGCF®-H (10-80 µg) by pharyngeal aspiration; dispersion medium (DM) was used as vehicle. At 1, 7 and 28 days post-exposure, lung lavage and histopathology studies were conducted. VGCF®-H cytotoxicity was assessed by measuring acellular lavage fluid lactate dehydrogenase (LDH) activity, and determined that VGCF®-H exposure produced dose-dependent increases in LDH activity which decreased over time. Using polymorphonuclear leukocytes as a marker, VGCF®-H exposure produced dose-dependent lung inflammation which decreased over time. Histologically, the incidence and severity of pulmonary inflammation was confirmed to be dose-dependent, and inflammatory infiltrates were characterized by increased numbers of alveolar macrophages with small numbers of neutrophils. VGCF®-H caused dose- and time-dependent increases in cathepsin activity and cytokines in the acellular lavage fluid, indicating activation of the NLRP3 inflammasome by VGCF®-H may contribute to lung inflammation. VGCF®-H exposure caused minimal to mild interstitial alveolar fibrosis, characterized by increased amounts of collagen fibers in the interstitium, and the incidence and severity of fibrosis tended to increase with VGCF®-H dose. Compared to our previous studies with MWCNT-7, on an equal mass basis, pulmonary inflammation and fibrosis appeared to be less severe in VGCF®-H exposed mice lungs.

Keywords: Carbon-based nanomaterials, Hazard ranking/characterization, In vivo toxicology, Occupational safety

193. Attenuation of the Adverse Health Effects of Metallic and Metal-Oxide Nanoparticles: Theoretical Assumptions and Experimental Data

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Especially high health risks associated with impacts of metallic nanoparticles (Me-NPs) and their presence in the working environments of not only the nanoindustry but also of some long-existing traditional technologies make it necessary, along with keeping respective dangerous exposures as low as possible, to look for ways of increasing the organism's resistance to them. Based on theoretical premises of such beneficial interference with toxicokinetics and toxicodynamics of Me-NPs developed by our research team and on understanding general and specific key mechanisms of different Me-NPs' toxic action, we proposed several bioprotective complexes (BPCs) comprising mainly pectin, some vitamins, glutamate, glycine, N-acetylcysteine, omega-3 PUFA, and different essential trace elements. Results of our "in vivo" experiments with NPs of silver, of copper oxide, and of manganese and nickel oxides in combination, showed that, against the background of such BPCs' oral administration, the integral and specific toxicity of Me-NPs and even their genotoxicity can be markedly attenuated. Therefore we would recommend to further develop this vector of nanotoxicological research. Our previous positive experience in organizing first a selective and then a large-scale "biological prophylaxis" of adverse health effects of many other toxicants makes us expect that it would be no less practicable and effective in the field of nanotoxicology as well.

Keywords: Biokinetics/toxicokinetics, In vivo toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms

194. Computational Nanotoxicology - Status and Perspectives

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Experimental in vitro and in vivo testing in nanotoxicology might be significantly supported by the application of computational modeling (in silico studies). Such strategy helps to reduce cost, time and the use of laboratory animals. Moreover, it is important in the context of recommendation of the EU REACH regulation and the 3Rs principle (Replacement, Reduction, Refinement of animal testing). In silico techniques can be divided into three groups, namely: (i) computational chemistry methods (Molecular Dynamics, Quantum Mechanics and Density Functional Theory calculations); (ii) chemoinformatic methods (Quantitative Structure-Activity Relationships modeling, similarity analysis and read-across) and (iii) bioinformatics methods. The presentation will discuss the current state-of-the-art including the examples of successful application of the in silico methods and future perspectives for the development of computational nanotoxicology.

Keywords: In silico modeling, computational nanotoxicology

195. Nanotechnology to the Rescue: A Chemical Free, Nanotechnology Based Antimicrobial Platform for Inactivation of Foodborne Microorganisms Across the "Farm To The Fork" Continuum Using Engineered Water Nanostructures (EWNS)

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Foodborne diseases caused by the consumption of food contaminated with pathogenic microorganisms have very serious economic and public health consequences. Here we present the effectiveness of a new intervention method for inactivation of food related microorganisms using Engineered Water Nanostructures (EWNS). EWNS are synthesized using an integrated process which is based on electrospraying and ionization of water. EWNS are 25 nm in diameter, remain airborne in indoor conditions for hours, contain Reactive Oxygen Species (ROS) and have very strong surface charge (between 10 - 40e/structure). The EWNS properties can be "fined-tuned" during their synthesis to optimize their microorganism inactivation potential. We explore their efficacy in inactivating representative foodborne bacteria such as Escherichia coli, Salmonella enterica, Listeria innocua, Mycobacterium parafortuitum, and Saccharomyces cerevisiae on organic cherry tomatoes using the moderate dose of 40,000 #/cm^3. The preliminary results presented here showcased that EWNS can achieve microbial removal rates between 1 and 3.82 logs after 45 mins of exposure. EM imaging revealed that the EWNS could destroy the bacteria membrane leading to their inactivation. The membrane destruction was due to the presence of ROS as shown with a lipid peroxidation assays. This scalable antimicrobial platform has can be used across the farm-to-fork continuum to enhance food safety, quality and minimize spoilage and food waste.

Keywords: Biomedical/therapeutic applications, Emerging nanomaterials, Green nanomaterials, Food, Agriculture

196. Influence of Surface Functionality on Apolipoprotein and Nanosilver Biocorona

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It is beginning to be recognized that formation of a 'biocorona' is critical to the biological interactions of engineered nanomaterials (ENMs). Upon introduction into a biological system, ENMs rapidly associate a variety of macromolecules including proteins, peptides, amino acids, fatty acids, lipids and other organic matter forming a biocorona. The formation of the corona is not only dictated by the physicochemical properties of the ENM but also the composition of the physiological environment. ENM-biocorona with apo-lipoprotein A1 (apoA1), a vital component of HDL, may increase the risk of myocardial infarction in patients with obesity. Here, we will present the influence of surface functional groups on Ag nanoparticles on the formation of apoA1 biocorona and the associated immune response. In particular, we show that the amount of protein adsorption and protein secondary structural changes are highly dependent on the surface functionality.

Keywords: Biocorona, Metal/metal oxide nanomaterials, Nanomaterial release

197. Evaluation of Vascular Tone and Cardiac Contractility in Response to Silver Nanoparticles, Using Langendorff Rat Heart Preparation

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Silver nanoparticles (AgNPs) have been widely used in an increasing number of applications because of their antimicrobial properties. However, AgNPs exposure can induce adverse responses in major organs, including the heart. Several reports suggest that AgNPs-induced cardiac effects involve nitric oxide (NO) generation, derived from NO synthases (NOS), as well as oxidative stress generation either by reducing antioxidant enzymes activity such superoxide dismutase (SOD) and catalase (CAT) or increasing reactive oxygen species (ROS) levels. Nevertheless, no studies about the AgNPs-induced effects in the cardiac physiology have been performed. The aim of this study was to evaluate the direct actions of AgNPs on coronary vascular tone and cardiac contractility using the isolated and perfused rat heart Langendorff preparation. AgNPs (15±4 nm) at 0.1 and 1 μg/mL induced a slight vasodilation and decreased cardiac contractility dependent on NO production. Meanwhile, high concentrations of AgNPs (10 and 100 μg/mL) promoted vasoconstriction and cardiac contractility related to the inhibition of endothelial NOS phosphorylation and inducible NOS expression as well as increased oxidative stress, without modifying SOD or CAT expression. Furthermore, AgNPs inhibited classic actions induced by phenylephrine (Phe) and acetylcholine (ACh), indicating that AgNPs may influence the effects of vasoactive and inotropic agents. These data suggest that AgNPs affect cardiac physiology through generation of NO, NOS expression and oxidative stress generation. Further investigations are required to elucidate the mechanism of action and signaling pathway involved in these events.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Toxicological mechanisms, Silver nanoparticles, coronary vascular tone, myocardial contractility, nitric oxide, oxidative stress

198. Comparative Evaluation of Acute and Chronic Ecotoxicity of Copper Oxide Nanoparticles on the Pond Snail Lymnaea Stagnalis

Valentina Ricottone, Vicki Stone, Theodore Henry, Teresa F Fernandes. Heriot-Watt University, UK.

Nanotechnology is a rapidly developing field in the 21st century, and the commercial use of nanomaterials for novel applications is increasing exponentially. Copper oxide nanoparticles (CuO NPs) are frequently employed for their antimicrobial properties in antifouling paints. Their extensive use can contaminate aquatic ecosystems. The objective of this study was to evaluate and compare the aquatic toxicity of CuO NPs through acute and chronic toxicity tests with different life stages of the snail Lymnaea stagnalis, a representative organism of the benthic ecosystem. Acute waterborne exposure was focused on the evaluation of the acute lethal toxicity of CuO NPs to juveniles (7-9 day old) of the pond snail L. stagnalis exposed for 96h at 20°C to Cu in a static experiment, either in the nano form of CuO NPs or ionic form, as $CuSO_4*5H_2O$. The LC_{50} value estimated in tests with $CuSO_4$ (LC_{50}96h = 5.7 μg L^{-1} Cu) was much lower than that obtained for the tests with CuO NPs (LC_{50}96h = 2500 μg L^{-1} Cu). Chronic toxicity tests aimed to investigate the effects of exposure CuO NPs on the reproduction to L. stagnalis. Young adult snails (22±2mm) were exposed to Cu as CuO NPs at 20°C for 30 days in a semi-static experiment. Endpoints such as: mortality, growth and behaviour alteration were also evaluated along with the reproduction parameters. LC_{10}30d and LC_{50}30d values estimated were respectively, 230 μg L^{-1} Cu and 480 μg L^{-1} Cu, indicating higher toxicity than the acute test. Additionally, exposure to CuO NPs showed significant effects on the growth and reproduction parameters relative to the control. Behavioural changes, such as avoidance and respiration behaviour, were also observed in the treatments. The experiments results demonstrate a time-related increasing toxicity of CuO NPs on L. stagnalis, emphasizing the need for more chronic study to accurately evaluate the impact of nanomaterials in the real environment. Acute and chronic tests assessing the toxicity of safe-by-design CuO NPs and their fragmented products (FP) on L. stagnalis will be performed, applying the same experimental design used for evaluate the toxicity of the pristine CuO NPs.

Keywords: Environmental nanotoxicology, Lymnaea stagnalis, Copper oxide nanoparticles, chronic exposure, reprotoxicity

199. Toxicological Evaluation of Graphene Nanomaterials That Differ in Size and Oxidative Form Following Pharyngeal Aspiration in Mice

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As manufacturing of various graphene nanoparticles (GNP) expands, there is concern for the health effects due to pulmonary exposure in workers. Studies were conducted evaluating pulmonary toxicity of GNP of different size and oxidative form. Mice were exposed by pharyngeal-aspiration to 4 or 40 mg of non-oxidized graphite nanoplates (Gr) of different sizes (20 mm lateral, 7-10 nm thick; 5 mm lateral, 7-10 nm thick; <2 μm lateral, 1-2 nm thick), an oxidized-intermediate similar in size to 5 mm Gr (GO), or the reduced form of GO (rGO; ~1-2 mm lateral dimension post-dispersion). Multi-walled carbon nanotubes (MWCNT) and carbon black (CB, 15 nm diameter) were particle controls. Lavage, histopathology, and RNA analysis were performed 4-hr, 1-d, 7-d, 1-m, and 2-m post-exposure. On a mass basis, the larger sizes of Gr were found to be more inflammatory compared to the smaller size. However, Gr, GO, and CB caused transient lung inflammation/injury when compared to rGO and MWCNT. 40 mg rGO also caused a pulmonary fibrotic response comparable to MWCNT. rGO is less dense and has smaller dimensions than Gr and GO; therefore, at the same dose, a greater particle volume/number was delivered to the lungs at the high dose of rGO compared to the larger Gr. rGO at 4 mg was more equivalent by volume to 40 mg of Gr particles, and did not produce persistent toxicity in the lung. Taken together, the data suggest that GNP chemistry may play a role in toxicity, however lateral size/aspect ratio may be more critical.

Keywords: Carbon-based nanomaterials, In vivo toxicology, graphene, pulmonary toxicology

200. Development of a Fish Liver Microtissue Model to Characterize the Toxicity of Aromatic Hydrocarbons and Nanoparticle-Based Dispersants

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Engineered nanoparticles can assemble at water-oil interfaces to stabilize oil droplets into an emulsion, and are under development for use as dispersants following oil spills. This project focuses on the potential impacts of environmental exposures to these nanoparticles on aquatic organisms. Using a fish liver cell line, PLHC-1, in a three-dimensional (3D), scaffold-free microtissue model, we are examining the environmental impacts of co-exposure to polycyclic aromatic hydrocarbons and surface-engineered carbon black as a model nanoparticle dispersant. 3D cultures can provide the benefits of tissue-like cell-cell interactions and the opportunity for longer term cultures, but also present challenges associated with imaging, maintaining viability, and adapting two dimensional assays to 3D systems. For this novel fish liver microtissue model, assays to determine the toxicity of aromatic hydrocarbons and nanoparticles, including biomarkers of stress and xenobiotic metabolism, were optimized in monolayer and then adapted for use in 3D with toxicants. To characterize the fish liver spheroids, changes in viability, differentiation, and response to toxicants were measured during long term cultures of spheroids. These results demonstrate that this 3D fish liver model is a highly responsive platform for the study of nanomaterials and additional toxicants. This research is supported by BP/The Gulf of Mexico Research Initiative, NIEHS Training Grant T32 ES07272, NIEHS Superfund Research Program P42 ES013660, the Institute at Brown for Environment & Society, and the generous support of Donna McGraw Weiss ’89 and Jason Weiss.

Keywords: Alternative testing methods/strategies, Environmental nanotoxicology, In vitro toxicology

201. Food Grade Titanium Dioxide Nanoparticles Have Photocatalytic Activity and Induce Genotoxicity

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Food grade titanium dioxide (E171) is widely used for its whiteness and opacity as an additive in food and personal care products, it is made of up to 36% nanoparticles which are particles less than 100 nm in diameter. E171 can reach the gastrointestinal system through the oral route and accumulate in many organs such as liver, spleen and colon. Since E171 has been poorly investigated, the aim of this work was to evaluate the E171 photocatalytic activity and its genotoxic potential. We performed a photocatalytic acellular assay to E171 particles consisting in the degradation of methylene blue (MB). Also we tested cell viability by trypan blue assay and genotoxicity by the micronucleus test in a colorectal cancer cell line (HCT116). HCT116 cells were treated with 5, 10 and 50 µg/cm^2 (50, 100, 500 µg/ml) of E171for 24 h. We found out that E171 can degrade MB but less than TiO_2 nanoparticles. Cell viability did not show significant changes after exposure to E171 for 24 h and the frequency of micronucleated cells increased in a 1.9, 2.4 and 3.6-fold manner corresponding to 5, 10 and 50 µg/cm^2 respectively. These results indicate that E171 has photocatalytic activity which hasn't been proved before, also E171 can cause DNA damage even when it doesn't affect cell viability. Further study is needed to understand the mechanism of E171 genotoxicity and improve the safe use of nanoparticles as food components. Funding was provided by Consejo Nacional de Ciencia y Tecnología (CB-2011/166727).

Keywords: Carcinogenicity, Genotoxicity, In vitro toxicology, Physicochemical characterization, Food nanomaterials

202. Impact of an Integrated In Vivo-In Vitro Approach for Evaluating the Hazardous Pulmonary Effects of Nanomaterials and the Underlying Mechanisms

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With the promising benefit of nanotechnology and the rapid rise of engineered nanomaterial production, potential human exposure to nano-scaled respirable particles has become a major concern. Animal exposure studies have shown that pulmonary exposure to nanoparticles, such as carbon nanotubes (CNTs) and nano-scaled cerium oxide ($nCeO_2$), can deposit particles deep in lung tissues and cause specific harmful effects. Unique physicochemical properties of the nanoparticles greatly influence their adverse bio-activities. With the identification of the specifically affected lung cells at the site of particle accumulation, we have developed multiple in vitro models to assess the cytotoxic, fibrogenic, and carcinogenic potential of nanomaterials using cultured human lung cells. All in vitro doses were physiologically relevant and based on in vivo doses that induced significant pulmonary disorders in animal models. Acute (days), sub-chronic (weeks), and long-term (several months) exposures to CNTs, $nCeO_2$, and nFe_2O_3 were shown to cause dose- and time-dependent cytotoxic (cell damage) and fibrogenic (collagen production) effects, as well as neoplastic and/or malignant transformation (anchorage-independent growth, apoptosis evasion, increased migration, invasion, angiogenesis, and tumor formation), consistent with the in vivo animal data. In vitro assessment tools further allow detailed mechanistic investigations of key signaling pathways and mediators involved in the pathologic processes (e.g. p53, transforming growth factors, and matrix metalloproteinases), which may serve as predictive biomarkers for the in vivo responses. Impact of the integrated in vivo-in vitro approach also includes that it supports the utility of the in vitro models as rapid screening tools for risk assessment of nanomaterial-induced pathologies.

Keywords: Carbon-based nanomaterials, Carcinogenicity, In vitro toxicology, Toxicological mechanisms

203. Cerium Oxide Nanoparticles Moderately Enhances the Cancer Features Exhibited by Cigarette Smoke Condensate in a Human Lung Epithelial Cell Line

Laura Rubio Lorente, Jordi Bach Griera, Ricard Marcos Dauder, Alba Hernandez Bonilla. UAB, Spain.

Cerium oxide nanoparticles (CeNPs) have gained in popularity due to their autoregenerative redox cycle, which confers radical scavenging properties. Accordingly, CeNPs have been considered in several studies as a promising antioxidant therapeutic agent for different oxidant diseases, including cancer. In this context, we assessed the protective role of CeNPs in an environmentally relevant in vitro scenario where human lung BEAS-2B cells were long-term coexposed for 6 weeks to low doses of CeNPs and Cigarette smoke condensate (CSC), a well-known carcinogen. The acquisition of an in vitro cancer-like phenotype was assessed to see the differential effects of CSC alone and CSC+CeNPs. Matrix metalloproteinase (MMP) activities were measured by zymography and promotion of anchorage-independent cell growth were evaluated by soft agar assay. Cellular morphology, proliferation and differentiation status were also included as complementary measures of transformation. Our results show that cells exposed to the highest dose of CSC exhibit features of a cancer phenotype as indicated by spindle-like cell morphology, increased proliferation, deregulated differentiation status, increased MMPs secretion, anchorage-independent cell growth and enhancement of tumour growth. Contrary to expectations, CeNPs co-exposure moderately enhances the cancer features exhibited by all CSC treatments, thus indicating that the CSC-associated transforming effects are certainly not prevented by CeNPs. Interestingly, expression studies with FRA-1 showed that its down-regulation may be an important mechanism of carcinogenesis associated to our transforming conditions. Our work points out the importance of long-term exposures when assessing the nanoparticles-associated risk, as its properties and effects may vary considerably depending on the exposure scenario.

Keywords: Alternative testing methods/strategies, Carcinogenicity, Cerium oxide nanoparticles

204. Synthesis, Catalytic, Antimicrobial and Cytotoxicity Evaluation of Gold & Silver Nanoparticles Using Biodegradable, Π-Conjugated Polyamic Acid

Omowunmi Sadik, Victor Kariuki, Idris Yazgan. SUNY-Binghamton, USA.

We hereby report a rapid, simple, and one pot synthesis of silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) using conductive, electroactive and biodegradable. Poly (amic)acid (PAA) polymer as both the reductant and stabilizer. The synthesized AgNPs and AuNPs were characterized using transmission electron microscopy (TEM), High resolution HRTEM, energy dispersive spectroscopy (EDS), X-ray diffraction (XRD) and ultra-violet visible spectroscopy (UV-Vis). UV-VIS spectra exhibit major peaks at 440nm and 535nm for AgNPs and AuNPs respectively. The XRD patterns revealed four diffraction peaks at 38.12˚, 44.07˚, 64.27˚, and 77.22° that can be indexed to the (111), (200), (220), and (311) planes of face-centered cubic (fcc) silver crystallites respectively. The size of the crystallites along the [111] direction was estimated to be 4.2±0.5 nm, which is in agreement with the TEM result. The effect of temperature on the formation of AgNPs in the presence of PAA was investigated and found to be significant at 100℃, resulting in silver-polyamic acid nanocomposite without altering the fcc crystal pattern. The prepared AuNPs and AgNPs were found to exhibit catalytic activity towards 4-nitrophenol and methylene blue with a rate constant of 5.2×10^{-3} S-1 and 1.09×10^{-2} S-1 respectively. Finally, the synthesized AgNPs exhibit excellent antibacterial activity against Gram negative (E coli DH5 Alpha, E.coli 25922, Aeromonas hydrophilia & Pseudomonas aeruginosa) and gram positive (Listeria monocytogenes strains F2365 and HCC7 and S. epidermidis) bacteria in addition to modest cytotoxicity against non-cancerous immortalized IEC-6 and cancerous Caco-2 cell lines.

Keywords: Emerging nanomaterials

205. Antibacterial Activities and Cytotoxicity of Green Synthesized Stable Gold Nanoparticles from Flavonoid Derivatives

Omowunmi Sadik, Francis Osonga, Idris Yazgan, David Luther, Phuong Nan. SUNY-Binghamton, USA.

The fundamental goal of sustainable nanotechnology is tailored towards nanoscale control of synthesis and processing of matter without footprints that give rise to environmental degradation. Currently there is a search for synthetic methods that utilize fewer amounts of materials, water, and energy; while reducing or replacing the need for organic solvents. We hereby present a novel approach for synthesis of gold nanoparticles using naturally-derived water soluble flavonoids including Quercetin pentaphosphate (QPP), Quercetin sulfonic acid (QSA) and Apigenin Triphosphate (ATRP) which were utilized both as reducing agent and stabilizer. The synthesis was achieved at room temperature using water as a solvent and it requires no capping agents. Hence the approach contributes immensely in promoting ideals of green synthesis and nanotechnology by eliminating the use of hazardous and toxic organic solvents and adopting the use of water as a solvent. The synthesized nanoparticles were characterized using Uv-visible spectroscopy (Uv-Vis), X-ray diffraction (XRD), Transmission electron microscopy (TEM), Energy dispersive absorption spectroscopy (EDS). The gold nanoparticles were spherical in shape with average particle size of 10.45 nm, 12.66 nm and 13.54 nm for the nanoparticles derived from QPP, ATRP and QSA respectively. The AuNPs exhibited excellent antibacterial activities which can be utilized in water purification.

Keywords: Green nanomaterials, Flavonoids, green synthesis, gold nanoparticles, antibacterial activity

206. Genome Wide Association Study Of Mast Cell Degranulation By Nanoparticles

Monica Sandoval-Johnson[1], Laura Saba[1], Alison Bauer[1], Ramakrishna Podila[2], Jared Brown[1].

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Silver nanoparticles (AgNP) are the most widely manufactured engineered nanomaterial (ENM) due to their antimicrobial properties and therefore have an increased prevalence of human exposure. Our laboratory previously determined that mast cells, which are central to the innate immune response, are activated following AgNP exposure and is dependent on key physiochemical properties such as size and shape. Genetics are a major contributing factor in many toxicological outcomes, however to date, few studies have examined the contribution of genetic in ENM toxicity. We hypothesized that in addition to ENM properties, genetic factors contribute to the regulation of mast cell degranulation following AgNP exposure. We grew bone marrow-derived mast cells from genetically diverse mouse strains, exposed them to 20nm citrate-coated AgNP (25ug/mL) and assessed degranulation. Quantitative trait loci (QTL) mapping was preformed to identify single nucleotide polymorphisms (SNPs) associated with variation in degranulation patterns following AgNP exposure. Mast cells grown from 23 genetically different strains displayed a wide range of degranulation patterns following exposure. This suggests that multiple genes are likely regulating the different responses leading to mast cell activation. QTL mapping identified 2 statistically significant loci associated with mast cell degranulation; rs33109340 on chromosome 1 and rs47581453 on chromosome 7. These results provide evidence that a complex set of genes regulate mast cell responses to ENM exposure. Overall, the proposed research will contribute to the field of nanotoxicology by identifying genetic targets that play a role in adverse immune responses to further understand underlying mechanisms of toxicity. Funding: NIEHS RO1 ES019311.

Keywords: Adverse outcomes pathway analysis, Metal/metal oxide nanomaterials, Bioinformatics

207. Risk Management: A Cost Effective Strategy For Commercializing Nanotechnology In the Face of Slow or Missing Regulation

Joseph Sano, Julie Barry. Prince Lobel Tye, LLP, USA.

The absence of clear, understandable and properly informed science-based regulation or consensus industry standards governing engineered nanomaterials or products containing engineered nanomaterials, presents management with poor visibility, and unique challenges in assessing substantial investments in nanomaterial or nanomaterial product commercialization. The result is an uncertain environment in which to assess the costs and benefits for product research and development, design, manufacture, scalability, distribution and sale. In the absence of such regulation or standards, the void is often complicated by non- governmental organizations ("NGOs") which may have agendas that may be counterproductive to commercialization of nanotechnology. The debate over regulation versus voluntary codes of conduct can be seen in the recent bans on use of "microbeads" in everything from cosmetics and other consumer products, to industrial uses. Enterprise Risk Management (ERM) is a sound basis to cope with this uncertain environment:

ERM requires "What if" thinking by senior management which starts with existential threats to the enterprise: a. What if my Nano-enabled product causes substantial harm to a large number of people?; b. What if insurers decide to effectively exclude nanotechnology risk from liability insurance policies?; c. What if the Government bans my product?; d. What if the Government imposes cost prohibitive manufacturing or product-safety controls?

The next step in effective ERM (after risks are identified) involves some combination of: Cost effective risk reduction; Cost effective risk transfer (contractual indemnity, insurance).

The Impact of Slow or Missing Nanotechnology Regulation on existential threats to Nano-Commercialization: a. Both the European "precautionary principle" model and the US model of study and learn, regulate when necessary, have their advocates and their critics regarding which approach is better protective of human health and the environment, and which approach is better to enhance Nano commercialization; b. Regardless of whether the US moves to the precautionary principle, or eventually imposes burdensome regulation, existential threats to enterprises seeking to commercialize Nanotechnology remain.

What can a small commercial entity trying to commercialize Nanotechnology do to mitigate these existential threats?: a. Follow the current science regarding EHS impacts of your Nanotechnology, related ENM's and Nano toxicology in general; b. Hire effective legal counsel to coordinate your risk reduction/risk transfer strategies: i. Review and expand to the extent feasible upstream and downstream contractual indemnifications; ii. Review insurance coverages and the adequacy of limits; iii. Understand and explain existing and potential liability risks; iv. Work with the enterprise (and its EHS professionals) and the regulators to develop and implement cutting edge EHS controls, and impact any eventual regulation; c. Engage with regulators and help them understand real world commercialization circumstances, and join with regulators in developing cost-effective regulation; i. One NENA Member's Story of effective nano-risk management in action.

Keywords: Commercialization, Regulatory decision making, Risk assessment

208. Understanding the Impact of Biocorona on the Interaction of Gold Nanoparticles with Human Blood Components

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Despite colloidal gold nanoparticles (AuNP) being proposed for a multitude of biomedical applications, there is a lack of understanding on how the protein corona (PC) formation over AuNP influences its interaction with blood components. This study evaluated 40 and 80 nm AuNP with three surface coatings; branched polyethyleneimine (BPEI), lipoic acid, and polyethylene glycol (PEG), exposed to pooled human plasma for 1, 6, 12 and 24h to study the time-dependent evolution of the PC using differential centrifugal sedimentation (DCS), dynamic light scattering and nanoparticle tracking analysis. This study investigated the impact of AuNP-PC interaction with human blood components by evaluating red blood cell aggregation, hemolysis, platelet activation, platelet aggregation, prothrombin time, activated partial thromboplastin time, cytokine release, complement activation, lymphocyte proliferation and immunosuppression. DCS and zeta potential analysis revealed instantaneous protein adsorption over AuNP which altered the NP size and surface charge. Irrespective of size or surface functionalization, plasma protein coated AuNP exhibited compatibility with RBC, platelets, lymphocytes and plasma coagulation pathways. For instance, PC formation over BPEI-AuNP alleviated RBC aggregation, hemolysis and platelet activation of positively charged BPEI-AuNP. More importantly, AuNP protein complex did not activate the complement system or cause any immune cell activation or immunosuppression. This study provides further insight on the formation and kinetics of the biocorona and its impact on the critical homeostasis of the blood, which has important implications in the safe development of long circulating stealth NP for future biomedical applications. The study was funded by Nanotechnology Innovation Center of Kansas State.

Keywords: Biocorona, In vitro toxicology, Gold Nanoparticles, Hematotoxicity

209. SODIS using Titanium dioxide nanoparticles: A positive aspect of nanotoxicity

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INTRODUCTION: In the Solar Disinfection (SODIS) method for purifying water contaminated with fecal coliform bacteria, the water is placed in clear PET (plastic) bottles painted black halfway lengthwise and exposing them to sunlight while placed lengthwise in a north-south direction. Purification occurs in about five hours. We have shown however that advanced oxidative methods using Titanium Dioxide nanoparticles can significantly reduce the purification time. METHOD: Titanium dioxide nanoparticles were deposited to a glass surface (1m x 1m) by a sedimentation followed by a baking process. This surface served as the active surface of a reactor which was tilted by an angle of 110 and exposed to sunlight. Contaminated water was allowed to flow by gravity down the surface of the reactor, recycled and tested for bacteria periodically. RESULTS: Using this method we were able to purify water at a rate up to 1 liter/min compared with 1 liter/5 hours of standard SODIS . CONCLUSION : This increased efficiency is attributed to the photocatalytic effect of TiO2 nanoparticles which together with u v light reacts with water to produce highly reactive hydroxyl ion species which kill the bacteria. ACKNOWLEDGEMENTS : We thank The Organization of American States and The University of the West Indies, St. Augustine, Trinidad, for grants.

Keywords: Methods

210. High Throughput Nanotox Screening Using Impedance: A Data Analysis Example

Alexander Sauter, Melanie Ostermann, Julia Schölermann, Ying Xue, Anne Burtey, Emil Cimpan, Mihaela Roxana Cimpan. University of Bergen, Norway.

A general problem in high throughput testing of nanomaterials is the control of the data quality, both concerning day-to-day performance and material-dependent interferences. Here, we show on an example of an impedance-based high throughput nano-cytotoxicity screening how uncharacteristic data can be detected for automatic exclusion, while allowing for a more sensitive analysis, revealing the fundamental dynamics in the biological processes. Further, identified material-dependent artifacts can be corrected for in order to preserve reliable toxicity information. Cytotoxicity results obtained by exposing 3 types of cells to 16 types of nanomaterials in 6 concentrations went into the analysis. The automated analysis makes use of growth similarities during an equilibration period prior to exposure, and an analysis of the typical cell growth in unexposed control conditions. The presented way of analysis ensures a more nuanced and reliable assessment of nanotoxicity, which is crucial for hazard estimation. The data was generated in the FP7 EU-project NANoREG and the NANO2021 project NorNANoREG.

Keywords: Hazard ranking/characterization, High throughput screening, In vitro toxicology, Methods,

211. Effects of SiO_2 and CeO_2 nanoparticles on Alzheimer-like pathology in mice after 3 and 14 weeks oral exposure

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There is an increasing concern about neurotoxic and neurodegenerative effects of engineered nanoparticles. We have investigated the effects of oral exposure to silicon dioxide and cerium dioxide nanoparticles on Alzheimer's Disease (AD)-like pathology in 5xFAD transgenic mice and their wild-type littermates. The mice were exposed ad libitum for 3 or 14 weeks to control feed pellets, or pellets enriched with either amorphous fumed SiO_2 (7 nm) or CeO_2 (NM-212, EU-JRC repository) at 1 mg/g and 10 mg/g feed. Following exposure, the mice were investigated for various AD-related features, including altered behaviour (i.e. string suspension test, X-maze, open field test), brain tissue homogenate levels of Amyloid-β (Aβ), and formation of Aβ-plaques in hippocampal and cortical brain regions. No major behaviour impairments could be observed in association with nanoparticle exposure in the transgenic as well as the wildtype mice. Treatment-related variations in Aβ40 and Aβ42 protein levels could be detected in the brain homogenates of the mice from both genetic backgrounds. However, immunohistochemical analysis revealed no accelerated formation of Aβ plaques in the nanoparticle-fed 5xFAD mice. The findings from present study suggest that long-term oral exposure to SiO_2 or CeO_2 nanoparticles may not have major adverse health impacts on the central nervous system, specifically regarding the development or progression of the neurodegenerative Alzheimer's disease. Supported by the EU (FP7 project NanoMILE) and the German Federal Ministry of Education and Research (BMBF, project N3rvousSystem).

Keywords: Cerium oxide nanoparticles, In vivo toxicology, Neurodegeneration

212. Efficient Detection of Nanomaterial-Cell Interactions by Flow Cytometry

Juergen Schnekenburger, Sarah Mues, Rainer Ossig. Biomedical Technology Center, University Hospital Muenster, Germany.

The in vitro cytotoxicity assessment of engineered nanoparticles is mainly endpoint driven. A more precise and specific nanomaterial toxicity testing requires a deeper understanding of the underlying cellular mechanisms and cell uptake pathways. We have investigated cytotoxic effects of spherical (NM 300) and rod shaped (NM 302) silver nanoparticles with a matrix of four cell lines representing different functions like lung epithelial cells, macrophages and fibroblasts on two different endpoints: cell viability (WST-8) and cell death (LDH). In addition we have used a flexible and label-free flow cytometer system to investigate the best detector-laser combination for analyzing cell-particle interactions and particle uptake by increased side scatter signals. Silver spheres lead to more cytotoxic effects than rods in all four examined cell lines and both assay. In contrast a dose dependent interaction increase of cells with NM 300 but even more with NM 302 analyzed by flow cytometry was detected. The significant differences of silver nanomaterials with various cell types demonstrate flow cytometry as valuable label-free tool for nanomaterial cell interaction studies. Optimized flow cytometry for nanomaterial cell interaction provides a rapid screen for industrial materials without any further labeling.

Keywords: Human toxicology, In vitro toxicology, Metal/metal oxide nanomaterials, Methods,Toxicological mechanisms, Nanoparticle cell interaction, Flow cytometry, Nanomaterial side scatter detection, Label free optical toxicity screening

213. Novel Multimodal Optical Quantification of Nanomaterial Cytotoxicity

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For many nanomaterials, especially materials with a low toxicity level, material assay interference inhibits the detection of regulatory relevant the low observed effect level (LOEL). Here we present Digital Holographic Microscopy (DHM) as a multimodal optical method, which overcomes the limitations of conventional in vitro assays. Using cell viability (WST-8) and cell death (LDH) assays as parameter we initially investigated the toxic effects of spherical (NM 300) and rod shaped (NM 302) silver nanomaterials with a matrix of four cell lines representing different organ functions. In addition, we applied DHM for multimodal label-free analysis of nanomaterial toxicity. Quantitative DHM phase images of cells were analyzed for refractive index, volume, density and dry mass. Silver spheres induced cytotoxic effects in all four examined cell lines compared to no toxicity of rods up to 10 µg/cm^2. Furthermore, we could correlate these data to a decrease of the cellular refractive index after incubation with NM 300 as well as a decreased dry mass and surface area development indicating reduced cell viability and cell death. DHM allowed us to increase the concentration of silver nano rods to the LOEL concentration. These results demonstrate the potential of DHM as novel valuable label-free tool for the analysis nanomaterial toxicity.

Keywords: Human toxicology, In vitro toxicology, Metal/metal oxide nanomaterials, Methods, digital holographic microscopy, label free optical toxicity screening

214. Novel Nano Inks for Additive Manufacturing - Establishing Nano Safety Risk Management Concepts in 3D Printing

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Additive manufacturing (AM) enables a new manufacturing paradigm, such as the rapid, distributive manufacture of complex 3D objects. In addition, AM will reduce waste and has the potential to produce custom-made products. Nanoparticles are in particular suitable for ink formulation of novel PolyJet inks to obtain functionalities embedded in the AM process. The DIMAP project ("Novel nanoparticle enhanced Digital Materials for 3D Printing and their application shown for the robotic and electronic industry") will advance the state-of-the art of AM through modifications of their fundamental material properties by using nanoscale material enhanced inks. This widens the range of currently available AM materials and integrates advanced functionalities. The main aim is to develop novel multi-material systems for PolyJet inks.Therefore, DIMAP will improve and advance the current technology by widening the range of available materials. Flame spray pyrolysis will be used in order to prepare different nanoscale oxide materials as fillers for inks, metallic nanoparticle inks will be developed for enabling PolyJet manufactured parts the functionality of electrical conductivity. DIMAP also targets the development of high strength polymeric material. However, the impact and interaction of nanomaterials on environment and human health is widely discussed today. Within DIMAP (nano)material characteristics will be documented in safety data sheets to ensure transparency and safe handling by all project partners. A concern-driven guidance for potential risk investigation will be established and assessment of the exposure rates during manufacturing, usage and end of life will be carried out. DIMAP is funded under EU H2020-NMP-PILOTS-2015, GA 685937.

Keywords: Emerging nanomaterials, Human toxicology, Nanomaterial release

215. Methodological Considerations for In Vitro, Air-Liquid Interface (ALI) Exposures to Engineered Nanoparticle Aerosols

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Dosimetry is an important aspect of exposure studies and can be subdivided into three metrics: administered dose, what is applied to the system, deposited dose, what adsorbs to and enters the cells, and the cellular dose, what enters the cells. Linking administered dose to deposited dose is deposition efficiency, which for in vitro ALI experiments, is highly dependent upon the exposure system used and the material tested. A systematic review of published ALI studies testing engineered nanomaterials revealed that there is no standardized experimental method and few systems are suitable for measuring aerosols on-site. We determined the need for a small, portable device for use at the source of particle emissions and have designed a Portable In Vitro Exposure Cassette (PIVEC). The PIVEC adapts the well-known 37mm filter cassette to contain a transwell for investigation of gases and particles at the ALI. Filter cassettes are widely used by industrial hygienists providing familiarity and ease in testing, as correlations between filters and in vitro experiments can expedite analysis. Through deposition experiments using saline and fluorescein, particles in the nano and micron range deposit in the PIVEC. Biological responses of lung cells to engineered nanoparticles will also be presented. The PIVEC will aid in the measurement of biological response to nanoparticle aerosols in different environments.

Keywords: Dosimetry, In vitro toxicology, Methods, Exposure systems

216. Cellular Effects of Al-, Ti- and Zn-Containing Nanomaterials on Intestinal Cell Lines In Vitro

Holger Sieg[1], Linda Boehmert[1], Caroline Lehmann[1], Claudia Kaestner[2], Benjamin Krause[1], Dajana Lichtenstein[1], Agnes Burel[3], Soizic Chevance[4], Jutta Tentschert[1], Peter Laux[1], Albert Braeuning[1], Andreas Thuenemann[2], Fabienne Gauffre[4], Irina Estrela Lopis[5], Valerie Fessard[6], Andreas Luch[1], Alfonso Lampen[1].
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Aluminum-, titanium- and zinc-containing chemicals are highly abundant in food, food contact materials and consumer products. Physical and chemical conversion might lead to a certain amount of nanoscaled particles that can be taken up by the gastrointestinal tract. Nanospecific effects such as higher reactivity, increased surface or altered uptake can increase hazardous potential for human health. The aim of this study as part of the European SolNanoTOX project is to characterize toxicological effects of Al-, Zn- and Ti-containing nanomaterials on intestinal cell lines. While toxicological potential of zinc species has been well studied, little is known about the effects of aluminum- and titanium-species. We have performed toxicological experiments on the human intestinal cell line Caco-2 for numerous endpoints: Cellular ATP and glutathione levels, apoptosis, necrosis, vesicular uptake, oxidative stress, growth rate and cell cycle modification. While zinc-containing controls showed toxic responses, our utilized aluminum- (elementary Al, γ-Al_2O_3) and titanium-species (TiO_2, rutile) did not. Nevertheless, we detected some differences between both different aluminum nanoparticle species and aluminum ions with regard to cell viability. We also provide strong evidence for particle-specific uptake of aluminum and titanium in the intestinal cell line Caco-2. In summary, among the different tested endpoints, Al- and Ti-containing nanomaterials did not show any toxicity in intestinal cell lines in vitro. Nevertheless, this absence of effect was not due to an absence of exposure, since particle-specific uptake was reported. Metal particle uptake over a long time might therefore be relevant for risk assessment of aluminum- and titanium-containing food products.

Keywords: In vitro toxicology

217. Toxicological Effects of Artificially Digested Al-Containing Nanomaterials on Intestinal Cell Lines In Vitro

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Although aluminum is one of the most common elements in the biosphere, little is known about its impact on human health. Since aluminum derivatives are highly abundant in food its oral uptake route is of toxicological relevance. Recently aluminum containing nanomaterials are considered to be linked to cancer and neurodegenerative disorders. Within the frame of the European SolNanoTOX project, we therefore investigated the toxicological effects of Al-containing species in different intestinal cell lines that represent the first biological barrier for food components prior to systemic distribution. In our in vitro digestion system, nanomaterials have been exposed to different physiological, chemical and biochemical conditions characteristic for saliva, gastric juice and the intestinal fluid. In vitro toxicity assays and cellular impedance measurements demonstrated the absence of cytotoxic effects of nanoparticles during a period of 48h after incubation. This was also observed after the digestion procedure. In contrast, aluminum ions from high concentrations of $AlCl_3$ showed larger effects on cell viability after the digestion procedure. In summary, the toxicological potential of aluminum-containing nanoparticles and ions to healthy intestinal cells appears to be low. Artificial digestion of these particles does not increase their toxic potential. Only for high doses of ionic aluminum, an increase of toxicity after artificial digestion was observed. Hence, we suggest that the release of Al ions from nanoparticles may lead to toxicity. Due to these observations, other cellular effects of Al-containing nanomaterials are required to be investigated.

Keywords: In vitro toxicology

218. Impact of (Artificial) Digestion on Al-Containing Nanomaterials and Their Physicochemical Characteristics

Holger Sieg[1], Linda Boehmert[1], Claudia Kaestner[2], Benjamin Krause[1], Dajana Lichtenstein[1], Agnes Burel[3], Soizic Chevance[4], Jutta Tentschert[1], Peter Laux[1], Albert Braeuning[1], Andreas Thuenemann[2], Fabienne Gauffre[4], Irina Estrela Lopis[5], Valerie Fessard[6], Andreas Luch[1], Alfonso Lampen[1]. [1]German Federal Institute for Risk Assessment; [2]German Federal Institute for Materials Research and Testing; [5]Institute of Medical Physics and Biophysics, University of Leipzig, Germany; [3]Mric-TEM BIOSIT, Université de Rennes; [4]Institut des Sciences Chimiques de Rennes; [6]ANSES, French Agency for Food, Environmental and Occupational Health Safety, France.

Aluminum and its chemical derivatives are highly abundant in food, food contact materials and consumer products. Up to now little is known about its derivatization and uptake during digestion and its impact on human health. As part of the SolNanoTOX project, different aluminum species were investigated during an artificial digestion process that mimics the saliva, the stomach and the intestine regarding pH-values, duration time, chemical environment and enzymatic composition. Two different nanomaterials (Al, Al_2O_3) and a soluble ionic $AlCl_3$ control were digested and investigated by different analytical methods regarding core radius, hydrodynamic diameter, agglomeration and dissolution behavior in biological media. The fate of nanoparticles during typical pH-values of saliva, gastric and intestinal juice was studied with dynamic light scattering (DLS), small angle X-ray scattering (SAXS) and ICP-MS in the single particle mode. After disappearance at pH 2 the nanoparticles were detected again in the intestinal fluid, as measured by DLS. During all artificial digestion stages Al nanoparticles had a constant average SAXS radius. In contrast, the radii of Al_2O_3 nanoparticles changed concentration-dependently. Highest radii were observed in the stomach fluid while intestinal fluid was found to cause full recovery of the primary particles. Dissolution of digested nanoparticles in cell culture media showed a bimodal size distribution of primary particles and aggregates. In summary, simulation of the gastrointestinal tract, mainly the change of pH settings, has provided evidence that the bioavailability of Al is likely to increase during the passage of the gut after oral uptake of aluminum-containing food products.

Keywords: In vitro toxicology

219. A 3D Multi-Cellular In Vitro Model for Study of Respiratory Toxicity of Particulate Matter

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Exposure to air pollution has the potential to induce severe adverse topic and system effects on human health, such as endothelial dysfunction or atherosclerotic malady. Despite the connection between air pollution and cardiovascular disease is well established, the underlying mechanisms, of which ROS production appears to be the key mechanism, are still unclear. We investigated the secondary toxicity effects induced by Diesel Exhaust Particulate Matter (DEPM) on the respiratory system by using a 3D multicellular in vitro alveolar model exposed realistic concentration (between 40 and 240 ng/cm2) of a naïve aerosol of DEPM, representative for a 24 hours exposure. The alveolar model used in this work is based on a combination of 4 different cell lines (A549; EA.ey926, THP-1 and HMC-1) grown at the Air Liquid Interface (ALI. Endothelial secondary toxicity was evaluated by measuring relevant endpoints such as nuclear translocation of the transcription factor Nrf2 and its downstream effectors together with metabolic activity, cell viability, and oxidative stress. Despite the low administered dose, it was possible to monitor the nuclear translocation of the nuclear receptor Nrf2 on the endothelial side of the 3D multicellular in vitro alveolar model. Results indicates that that the proposed model could be a valuable tool for the in vitro study of sub-acute doses and long term secondary effects of particulate matter.

Keywords: Carbon-based nanomaterials, Environmental nanotoxicology, In vitro toxicology, Toxicological mechanisms

220. Toxic Effects of Copper Sulphate And Copper Oxide Nanoparticles on Detoxification Enzymes of Galleria Mellonella

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Nanoparticles (NPs) are increasingly being used in biological and medical area. Therefore, their potential toxic effects resulting from use or unintentional release into environment. Metal-containing NPs comprises the largest number of NPs, which includes oxides such as zinc oxide (ZnO), titanium dioxide (TiO_2), cerium dioxide (CeO_2), copper oxide (CuO). Among the metal oxides, CuO NPs are two of the most commonly used NPs, and also are mostly applied in several products as skin products, and textiles mainly due to their antimicrobial properties. Acetylcholinesterase (AChE) is a key enzyme catalysing the hydrolysis of the neurotransmitter, acetylcholine, in the nervous system and is primarily responsible for termination of cholinergic neurotransmission at synapses in both humans and insects. Insect detoxifying enzymes, glutathione-s-transferase (GST) in detoxification of xenobiotics, protection from oxidative damage and intracellular transport of hormones, endogenous metabolites and exogenous chemicals. Accordingly, this study assessed the effects of copper oxide nanoparticles (CuO NPs) on AChE and GST activity using Galleria mellonella as bioindicators. To understand the effects of CuO NPs, third instar larvae were exposed to a realistic environmental concentration of 10 $\mu g.L^{-1}$ of $CuSO_4$ and CuO NPs to the last instar larvae. $CuSO_4$ and CuO NPs induced some physiological changes in AChE and GST activities of fat body and midgut of G. mellonella larvae. In conclusion, it is necessary to further improve the knowledge of NPs chemical behaviour, transport in and between environmental and biological compartments, ultimate environmental fate, mechanisms of biological uptake and toxic implications for living systems.

Keywords: Environmental nanotoxicology, Metal/metal oxide nanomaterials, Risk assessment, Acetylcholinesterase, Copper Oxide Nanoparticles, Galleria Mellonella, Glutathione-S-Transferase

221. Theory and Experiment: The Synthetical and Anion Binding Property of Tripodal Nano-Materials

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The study of molecular probe is attracted more attention by scientists. However, the literatures containing probe and nano-material information are corresponding few. A series of colorimetric anion probes containing OH and NO_2 groups were synthesized. Fortunatelly, the nano-materials of the above compounds were prepared successfully. Their recognition properties toward various anions were investigated by naked-eye observation, UV–vis, fluorescence, 1H NMR titration spectra and theoretical investigation. The results indicated that several nano-materials containing electron withdrawing substituents showed high binding ability for AcO−. The host-guest complex formed through 1:1 binding ratio, concomitant with a detectable color changes during the recognition process. Theoretical investigation analysis revealed the intramolecular hydrogen bond existed in the structure of the probe and the roles of molecular frontier orbitals in molecular interplay. The current studies suggested that this series of nano-materials can be used as colorimetric probes for the detection of AcO−.

Keywords: Systems biology/toxicology, nano-material synthesis

222. Development of an In Vitro Test to Assess the Inhalation Toxicity of Nanomaterials

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Although multi-walled carbon nanotubes (MWCNTs) are being used in consumer products, current understanding of their potential to cause adverse lung effects following inhalation remains limited. In many regulatory jurisdictions, a subchronic in vivo test (e.g., OECD test guideline 413) may be required for respirable substances including MWCNTs; however, ethical and scientific concerns associated with this test led an international expert group to evaluate in vitro approaches to assess the inhalation toxicology of MWCNTs. Pulmonary fibrosis was identified as a key adverse outcome linked to MWCNT exposure and recommendations were made to design a predictive in vitro assay. Subsequently, development of an in vitro co-culture system using relevant lung cells exposed to MWCNTs at the air-liquid interface (ALI) while considering human-relevant dosimetry and NM life-cycle transformations was undertaken. Mono- and co-cultures of human cell-lines, including alveolar epithelial cells, fibroblasts, and macrophages were exposed in suspension to MWCNTs (Mitsui-7 dispersed in H_2O with 0.1% bovine serum albumin) at 0.005, 0.01, and 0.02 mg/mL to assess the pro-fibrotic response. No cytotoxicity or increase in pro-inflammatory cytokines was observed at either 24 hrs or 5 days following exposure of epithelial mono-cultures to 0.02 mg/mL of MWCNTs. This work is complemented by comparative studies using a reconstructed primary human alveolar tissue model (EpiAlveolarTM) exposed to MWCNTs at the ALI using the VITROCELL® Cloud system. The work is funded by PETA International Science Consortium and the long-term goal is to develop an in vitro testing strategy using human-relevant methods to predict the pulmonary toxicity of nanomaterials.

Keywords: In vivo toxicology, inhalation toxicity, multi-walled carbon nanotubes, MWCNTs, in vitro testing strategies, regulatory risk assessment, dosimetry, pulmonary fibrosis

223. Mechanistic Evaluation of Oxidant Generation and the Development of Inflammation after Pulmonary Exposure to Metal-Rich Welding Nanoparticles

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Welding generates incidental nanoparticles composed of cytotoxic metals (e.g., Cr, Mn, Ni, Fe). The goal was to use both in vivo and in vitro methodologies to determine the mechanisms by which welding nanoparticles may damage the lungs. To assess lung injury/inflammation, Sprague-Dawley rats were treated by intratracheal instillation (ITI) with 2.0 mg/rat of gas metal arc-mild steel (GMA-MS) or manual metal arc-stainless steel (MMA-SS) welding particles. At 1, 3, and 10 d, bronchoalveolar lavage was performed. To determine the cytotoxicity of the welding nanoparticles, RAW264.7 macrophages were exposed to MMA-SS or GMA-MS particles for 24 hr (0-50 mg/mL), and ROS generation and activation of inflammatory markers were assessed. Particle metal composition and size distribution were characterized by ICP-AES and MOUDI, respectively. MMA-SS (41% Fe, 29% Cr, 17% Mn, 3% Ni) and GMA-MS (85% Fe, 14% Mn) were arranged as chain-like agglomerates (MMAD: 240 nm) of primary nanoparticles (~20-50 nm). Lung injury (LDH) and inflammation (neutrophil influx) were increased with ITI MMA-SS treatment compared to saline control and GMA-MS. RAW264.7 cells treated with MMA-SS caused increases in ROS, lipid aldehyde (4-hydroxynonenal) production, and COX-2 protein/gene expression. MMA-SS exposure also increased in vitro and in vivo protein expression of Nrf2 and HO-1 and in vitro gene expression of Hmox1. Welding generates nanoparticles with different metal profiles depending on the process. Welders exposed to SS particles are at a greater risk due to the presence of specific metals (e.g., Cr, Ni), increased cytotoxicity, and enhanced oxidative stress. Funding provided by NIOSH927ZLEG.

Keywords: In vitro toxicology, In vivo toxicology, Metal/metal oxide nanomaterials, Occupational safety,

224. Genotoxicity of Pristine, Heat-treated and Nitrogen-doped Multi-walled Carbon Nanotubes at Occupationally Relevant Doses

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The unique physiochemical properties of multi-walled carbon nanotubes (MW) make occupational respiratory exposures likely. Nitrogen-doped MW (NDMW) material has been shown to be less inflammatory than pristine MW (PMW). PMW exposed to extremely high temperatures (HTMW) reduces their bioreactivity in acellular systems. To investigate genotoxicity of NDMW and HTMW compared to PMW we used two human lung epithelial cell types, an immortalized BEAS-2B and primary SAEC. All MW were necrotic in both cells at the 24 µg/mL dose. There was no effect on the cell cycle in BEAS-2B cells. All MW induced a significant G0/G1 phase block in SAECs after 24 hour exposure to 24 µg/mL and a G1/S phase block after 72 hour exposure to 2.4 µg/mL. Clonal growth in primary SAEC cells was increased by 0.024 and 0.24 µg/mL HTMW and NDMW and 2.4 µg/mL HTMW. Significant increases in mitotic aberrations were observed by exposure to all MW for 24 hours. Although, all MW were found to penetrate the nucleus. Penetrations were greatest for the PMW followed by HTMW and NDMW, respectively. Increased fragmentation of the centrosome and centromere were found from exposure to all MW indicating a possible mechanism of genotoxicity. A dose-dependent increase in aneuploidy was observed from exposure to all MWs. Additionally, insertions and translocations between chromosomes 1 and 4 have been examined through fluorescent in situ hybridization staining of SAECs exposed to all MWs. These data indicate that altering the physiochemical properties of MWs may reduce their genotoxic effect.

Keywords: Carbon-based nanomaterials, Carcinogenicity, Genotoxicity, In vitro toxicology

225. Toxicity of Silica Nanoparticles: Interferences with Inflammatory Signaling Cascades

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Background. Silica based nanoparticles (SNP) are increasingly used for technical or pharmaceutical applications. This includes their use as filler, desiccant, thickener for liquid dosage forms, or anticaking agent in powders. More recently, they were also proposed as nano-sized drug delivery systems or imaging probes. In turn, this has raised concerns about their safety and biocompatibility. Notably questions about cellular uptake, transcellular transport and potential toxic effects of SNPs at the level of cellular barriers - such as capillary endothelia in the central nervous system – hamper their application in clinics. Aim. Cell viability, ROS generation, apoptosis, and cytokine mediated signaling pathways were studied using different types of SNP to elucidate their toxicological potential. As an endothelial barrier model, immortalized human brain capillary endothelial cells (hCMEC/D3) were used. Results were compared to effects in primary human umbilical vein endothelial cells (HUVEC). Results. SNP with positive and negative surface charges were synthesized and characterized with respect to size, shape, zeta-potential and polydispersity. Cellular interactions were studied by confocal microscopy, flow cytometry, and proliferation assays. Cellular uptake and toxicity were a function of dose, surface charge and incubation time. Direct toxicity was addressed in terms of viability, oxidative stress and hemolysis. In addition, it was shown that SNPs were able to activate distinct inflammatory signaling cascades. We therefore conclude that interactions of SNP with endothelial cells bear the risk of inducing pro-inflammatory cellular reactions.

Keywords: Adverse outcomes pathway analysis, In vitro toxicology, Toxicological mechanisms, Silica Nanoparticles

226. Hepatotoxicity Assessment of Iron Oxide Nanoparticles on Male Wistar Rat

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Iron oxide nanoparticles (IONPs) have great potential for modernizing nano-medicine with its application in biomedical devices and delivery systems. However, the toxicological effects of NPs or any adverse effects on human health have still not been addressed adequately. Hence, Present study was to aim to evaluate the hepatotoxic potential of IONPs. To address the issue we have selected iron oxide nanoparticles. Male Wistar rat were taken and divided into four groups. Control group was intravenously injected with saline whereas other groups were treated with different doses of IONPs i. e. 7.5, 15 and 30 mg/kg bw once in a week for 28 days. To evaluate the toxic effects, various parameters i.e. AST, ALP, ALT, reactive oxygen species (ROS) and antioxidant enzymes estimation, TBARS level, LDH assay and comet assay were performed. Atomic absorption spectroscopy method was used to measure accumulation of NPs in liver. TEM and histopathological analysis were used to observe any morphological changes induced by NPs. Results showed that nanoparticles significantly increase the activity of hepatic enzymes, LDH level, alter antioxidant enzymes and induce DNA damage at high dose. Bioaccumulation study shows that accumulation of IONPs in liver was increased significantly in a dose dependent manner. Pathological and ultrastructural changes also have been observed in liver. It may be concluded that IONPs get accumulated in liver and alter biochemical parameters, thus increased oxidative system, alter antioxidant system, and could induced histopathological and ultrastructural changes in liver which, affect the normal functioning of rat. Funding Source- UGC INDIA.

Keywords: Biodistribution, Environmental nanotoxicology, Genotoxicity, In vivo toxicology, Metal/metal oxide nanomaterials

227. Interaction of Nanoparticles with the Rat Alveolar Epithelial Monolayer (RAECM): Mechanisms of Uptake/Egress and Intracellular Fate of Nanoparticles

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We reported that polystyrene nanoparticles (PNP) are taken up from apical fluid of RAECM in a manner dependent on time, dose and particle size via a non-endocytic 'diffusional' process, while PNP egress from RAECM appears to take place via both a fast lysosomal-exocytosis and a slower 'diffusional'-process. In this study, we investigated involvement of autophagy in intracellular PNP (PNPic) fate. RAECM were exposed apically to carboxylated 20 nm PNP. [PNPic] was analyzed in live single cells by confocal microscopy. Uptake/egress kinetics of PNP were determined in the presence/absence of nocodazole or 3-ethyladenine (3MA). Cellular distribution/colocalization of PNP was studied by transducing LC3-GFP into RAECM to label autophagosomes, while lysosomes were stained using LysotrackerGreen. PNPic 24hr post-exposure were primarily localized in lysosomes. PNPic colocalized with LC3-GFP. Interference with autophagosome formation by 3MA resulted in decreased uptake and ~75% reduction in steady state [PNPic] without affecting PNP egress kinetics. Blocking microtubule polymerization with nocodazole blunted the Ca^{2+}-dependent fast egress of PNP.These data indicate that autophagy is involved in cellular processing of PNP in RAECM and appears to influence PNP uptake. Over time, PNP accumulate in lysosomes via autophagic flux, followed by subsequent fast release via exocytosis in parallel with the slower 'diffusional' egress process. Modulation of these processes may regulate [PNPic] and be important in protection from cellular injury by nanomaterials.

Keywords: Biokinetics/toxicokinetics, Nanomaterial release, Systems biology/toxicology, nanoparticle fate, autophagy

228. Reassessing Toxicity of Engineered Food-Grade Nanomaterials in C2bbe1 and Oral Keratinocytes

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Engineered food-grade nanomaterials (eFNMs) are currently being used as whitening, thickening, and coloring agents as additives in food. Although they are generally assumed to be safe, nanotoxicology on this class of nanomaterials is limited and the topic is generally understudied. Existing nanotoxicity studies have not utilized adequate dispersion and dosimetry protocols, and often times have used surrogate non-food-grade nanomaterials. Furthermore, characterization of eFNMs in digestive and cell culture media has been limited. The objective of the current study is to assess the impact of dosimetry of eFNMs on in vitro toxicity in representative cell lines of the oral cavity (HOK–human oral kertinocytes) and intestinal epithelial cells (C2BBe1–clone of Caco-2). Comprehensive physicochemical and morphological characterization of acquired eFNMs was accomplished using multiple complementary techniques – SF-ICP-MS, TEM, XRD, XPS, FTIR and, BET. Using standardized protocols, eFNMs were dispersed and characterized in DI water and cell culture media for morphology, size distribution and polydispersity index. Advanced computational model developed at Harvard (Distorted Grid model) was used to estimate in vitro dosimetry over time in cell cultures representative of the digestive system (C2BBe1 and HOK). The poster will present data on in vitro dosimetry of eFNMs in cell culture media including implications of these findings on the current understanding and validity of in vitro nanotoxicity of eFNMs.

Keywords: Dosimetry, In vitro toxicology, Physicochemical characterization

229. Formation and Characterization of Protein Corona Around of SiO2-PEG-Tf NP and Its Implications in Cellular Uptake.

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Active targeting is a promising therapy against cancer and nanoparticles are of interest for their application in nanomedicine, especially SiO_2 nanoparticles given their versatile functionalization. It has been suggested that NP functionalized under in vitro conditions would be covered by proteins from serum intervining with their cellular uptake. This could impair their targeting for treatment and favour their accumulation in undesired organs as well as promote adverse responses. The aim of this study was to synthesize SiO_2-PEG-Tf and characterize the protein corona in plasma in order to determine its implications for cellular uptake. We synthetized fluorescence SiO_2 NP with primary size of 26 nm, hydrodynamic diameter of 20-50 nm, PDI<0.3 and zeta potential from -21 to 31 mV in water. Their composition was corroborated by EDS. After the functionalization process with polyethylene glycol and transferrin, a thermogravimetric analysis showed important loss of mass (26.31% and 9.32%) at 289.22°C and 414.71°C corresponding to degradation of organic compounds. Protein content was quantified by BCA assay (80 µg prot/mg NP) and endotoxin level was <0.01 EU/ml by LAL assay. It was observed that protein corona formation decreased cellular uptake in A459 cells. Also the most abundant adsorbed proteins were in the range of 100 to 60 kDa and to a lesser extent high MW proteins. We are working on a proteomic analysis, in order to determine the relationships between the adsorbed proteins and physicochemical characteristics.This information will help to improve targeting strategies for safer nanotherapeutics. Source of funding: CONACYT, MX.

Keywords: Biocorona, Biomedical/therapeutic applications, Metal/metal oxide nanomaterials

230. Histopathological Aspects of Adult Zebrafish (Danio Rerio) After Chronic Exposure to Graphene Oxide

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Graphene exhibits unique physical and chemical properties, which have allowed a number of applications in different fields, including electronics and biomedical areas. However, the use of graphene could result in accumulation in aquatic environment, where the risks for organisms are still unknown. In this study, Danio rerio were exposed to 0; 2; 10 or 20 mg L^{-1} of graphene oxide (GO) during 14 days for assessment of effects on gill and liver tissues. After exposure, fish were collected and tissues samples were fixed, embedded in paraffin, and sectioned. Graphene oxide caused gills with dilated marginal channel, lamellar fusion, clubbed tips, mucocytes swollen, epithelial lifting, aneurysms and necrosis. Liver tissues exposed to GO presented some lesions, such as nuclei peripherally located. The hepatocytes exhibited a non-uniform shape, picnotic nuclei, vacuoles formation, rupture and necrosis. Graphene oxide nanoparticles are small enough to pass through the secondary lamellae of the gills, inducing significant alterations in these cells. Structural damages may bring vulnerability to the fish, especially related to osmoregulation and may also induce respiratory difficulties. The lesions on liver tissues could induce metabolic damages leading to cytotoxicity. In conclusion GO chronic exposure caused damages on gills and liver tissues structure of zebrafish.

Keywords: Biokinetics/toxicokinetics, Environmental nanotoxicology, In vivo toxicology, Risk assessment, Fish, Aquatic exposure, Histopathology, Graphene

231. Pulmonary Toxicity of Cuo Nanoparticles Using the Short-Term Inhalation Study Design

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Copper oxide nanoparticles (CuO NPs) are used in catalysts, solar cells, wood protection, electronics, antimicrobial products, inks and coatings in food packaging. Due to the potential for release of NPs into air, especially in an occupational setting, the inhalation route remains of interest. Here rats were exposed nose-only to a single exposure concentration, but for varying times each day for a total of 5 days in order to generate different dose levels of 0, 0.6, 2.4, 3.3, 6.3 and 13.2 mg/m^3. Primary particle size was 10 nm while MMAD was 1.5 µm. On day 6, dose-dependent lung inflammation and cytotoxicity was observed. Histopathological examinations indicated alveolitis, bronchiolitis, vacuolation of the respiratory epithelium and emphysema in the lung starting at 2.4 mg/m^3. After a recovery period of 22 days, limited inflammation was observed at the highest dose. The olfactory epithelium in the nose degenerated at 6.3 mg/m^3, but this was restored after 22 days. No histopathological changes were detected in the brain, olfactory bulb, spleen, kidney and liver. In conclusion, inhalation of agglomerated CuO NPs resulted in a dose-dependent toxicity in rats which almost fully disappeared following a 3-week post-exposure period.

Keywords: Biokinetics/toxicokinetics, In vivo toxicology, Metal/metal oxide nanomaterials

232. Developing Nanomaterial Treatments for Treating Tuberculosis

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Antiobiotic resistance for diseases such as Tuberculosis (TB) is a significant problem, with few drugs available to target the causative agent Mycobacterium tuberculosis and drug resistance on the increase. Nanomaterials (NM) offer the opportunity, through their antimicrobial properties and their ability to target phagolysosomes of macrophages to provide an exciting opportunity to develop new antibiotics. This study employed a rapid, low cost assay based upon a Green Fluorescent Protein reporter strain of Mycobacteruim avium subspecies paratuberculosis to screen the efficacy of metal (Ag), metal oxide (Cu(II)O and ZnO) and solid drug nanoparticles (SDN) based on FDA approved drugs (rifampicin, isoniazid and pyrazinamide). The screen generated results within 7 days which is a significant improvement on standard methods which take approx. 7 weeks. Mycobacterial sensitivity to the NP was found to be NP composition specific. The SDN were more potent than the metals/metal oxides and the equivalent solubilised drug. Combining drugs within a single SDN formulation enhanced further efficacy. For the metals/metal oxides toxicity was ranked: Ag> Cu(II)O> ZnO. Fluorescently labelled versions of the SDN colocalised with the mycobacterium within macrophages in vitro, and this colocalisation resulted in decreased viability of the mycobacterium demonstrating their efficacy within target cells. In conclusion the rapid in vitro screen developed provides an ideal opportunity to screen new antiobiotics for TB, and the SDN of existing FDA drugs enhance the efficacy of these drugs, probably via enhanced uptake and targeting of the mycobacterium.

Keywords: Biomedical/therapeutic applications, Composite nanomaterials, High throughput screening, nanomedicine, antimicrobial

233. The Essential Elements of a Risk Governance Framework for Current and Future Nanotechnologies

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Societies worldwide are investing considerable resources into the safe development and use of nanomaterials (NMs). While each of these efforts are crucial, individually they are insufficient and so the challenge is to develop a framework to coordinate the wide variety of actors and to facilitate consideration of the complex issues that occur in this rapidly evolving environment. Here we identify and describe three sets of essential elements required to generate an effective risk governance framework for NMs: 1. advanced tools for assessing the needs of users linked to risk assessment, mitigation and transfer which all feed into risk decision making; 2. an integrated model of likely human behaviour and decision-making; 3. legal and other (nano-specific and general) regulatory requirements to ensure compliance and to stimulate proactive approaches. Such an approach will systematically facilitate and incentivise good practice for the various stakeholders to allow the safe and sustainable future development of nanotechnology.

Keywords: Hazard ranking/characterization, Regulatory decision making, Risk assessment, Risk Governance, Risk Decision making

234. Nano-Ferric Oxide Induced Neoplastic-Like Transformation in a Human Primary Cell Model: Iron Homeostasis Disruption?

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As incorporation of engineered nanomaterials (ENMs) into technologies rise, the potential for long-term inhalation exposures is expected with largely unknown outcomes. Past ENM toxicity assessment has focused on acute exposures, with little attention paid to ENM-associated tumorigenesis. This study evaluated the use of human primary small airway epithelial cells (pSAEC) to serve as a Tier I neoplastic transformation screening model for ENM risk assessment. Low passage pSAECs were continuously exposed in vitro to 0.6 µg/cm^2 of nano-sized cerium oxide ($nCeO_2$), ferric oxide (nFe_2O_3), or multi-walled carbon nanotube (MWCNT Mitsui 7; 0.06 µg/cm^2) for 6 and 10 weeks. Vehicle-exposed cells served as passage controls. Exposed cells were evaluated for several cancer hallmarks to evaluate neoplastic transformation potential. At 10 weeks, nFe_2O_3-exposed cells displayed significant enhanced proliferation, invasion, soft agar colony formation and colony forming unit ability suggesting a neoplastic-like transformation. MWCNT-exposed cells exhibited increased colony formation ability while $nCeO_2$ was negative in all assays except proliferation. Next, nFe_2O_3 and MWCNT soft agar colonies were isolated and placed in culture to evaluate transformed phenotype longevity. All isolated clones, along with nFe_2O_3-exposed cells, maintained their transformed phenotypes, even after repeated (12-30) passages and freezing. Further studies suggested that potential disruption to iron homeostasis gave rise to increased intracellular iron and ROS production which is well-known to cause oxidative damage and promote cell transformation. Sub-chronic in vitro exposures with cancer hallmark screening using human pSAECs holds promise as a Tier I screening model for ENM-associated tumorigenesis. Funding: NIOSH NTRC, NIH R01-ES022968, R01-EB018857, NSF CBET-1434503.

Keywords: Alternative testing methods/strategies, Carcinogenicity, In vitro toxicology, Metal/metal oxide nanomaterials, Cell transformation

235. Autophagy and Autophagy Dysfunction Contribute to Apoptosis in HepG2 Cells Exposed to Nanosilica

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Great concerns have been gained to evaluate the potential hazards of nanosilica to human health and environment. However, there still exists persistent debates on biological effects and toxic consequences induced by nanosilica. The present study investigated both autophagy and apoptosis in ICR mice and Human hepatocellular carcinoma cells (HepG2), and then explored the interactive mechanism between these two distinct cell death modalities in HepG2 cells. The mice liver injuries exhibited in hematoxylin and eosin (HE) staining indicated hepatotoxic effects of nanosilica. The TUNEL assay and immunohistochemistry results confirmed that nanosilica could induce both apoptosis and autophagy in vivo. Flow cytometry analysis demonstrated apoptosis induction in vitro, while autophagic ultrastructures, LC3-II expression and immunofluorescence clarified autophagy activation by nanosilica. Apoptosis suppression by autophagy inhibitor of 3-Methyladenine (3-MA) implied that autophagy was involved in apoptotic cell death. Mechanistic study verified that nanosilica induced autophagy via negatively regulation of mammalian target of rapamycin (mTOR) signaling but not Beclin-1 associated pathway. The enhancement of p62 accumulation and mTOR down-regulation might account for the molecular mechanism in contribution of autophagy to apoptosis. As an emerging new mechanism of nanomaterial toxicity, autophagy might be a more susceptive indicator for toxicological consequence evaluation in nanoparticle toxicity. The present study provides novel evidence to elucidate the toxicity mechanisms and may be beneficial to more rational applications of nanosilica in the future.

Keywords: Emerging nanomaterials, Nanosilica, Cell death, Autophagy, Apoptosis

236. Contribution of Some Physiological and Physicochemical Mechanisms Controlling Pulmonary Re-Tention, Body Distribution and Elimination of Iron in Rats under Long-Term Inhalation Exposure to Fe_2O_3 Nanoparticles (Experimental And Mathematical Modeling)

Marina Sutunkova[1], Boris Katsnelson[1], Larisa Privalova[1], Vladimir Gurvich[1], Ludmila Konysheva[1], Vladimir Shur[2], Ekaterina Shishkina[2], Ilsira Minigaliyeva[1], Svetlana Solovjeva[1], Svetlana Grebenkina[1]. [1]The Medical Research Center for Prophylaxis and Health Protection in Industrial Workers; [2]The Ural Center of the Shared Use “Modern nanotechnologies”, The Ural Federal University, Russia.

Airborne Fe_2O_3 nanoparticles with the mean diameter of 14±4nm produced by sparkling from 99.99% pure iron rods were fed into a nose-only exposure tower. Rats were exposed to them 4 hrs a day, 5 days a week during 3, 6 or 10 months at the mean concentration 1.14 ± 0.01mg/m^3. An analogous appliance was used for sham exposures. Nanoparticles collected from the inhaled air proved insoluble in de-ionized water but dissolved markedly in the broncho-alveolar lavage fluid and in the bovine blood serum. The total iron and the iron of Fe_2O_3 (thus, presumably of particulates) content of different tissues and excreta were measured by AES and EPR methods, respectively. We found relatively low but significant pulmonary accumulation of Fe_2O_3 gradually increasing with time, while it was not detectable above control levels in blood, kidneys, spleen, liver and brain. However, we obtained TEM-images of nanoparticles penetrating into the anterior brain associated with ultrastructural damage to its neurons. A significantly increased concentration of Fe_2O_3 in feces testifying for the transfer of NPs from airways to the GIT was observed only after the 3 months exposure. We proposed a multicompartmental model describing toxicokinetics of inhaled nanoparticles after their deposition in low airways as controlled by their (a) high ability to penetrate through bio-membranes; (b) active endocytosis; (b) in vivo dissolution with resulting distribution and elimination patterns characteristic of the specific metal ions (free or protein-bound). However, in our case, just dissolution-depending mechanisms proved dominating due to rather high solubility of small Fe_2O_3-NPs in biological milieus.

Keywords: Biodistribution, Biokinetics, inhalation exposure

237. Beryllium Oxide Nanoparticles Interact with HLA-DPB1-positive Clara Cells

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Beryllium is among the light metals and is used in industries such as the automotive, electronics, aerospace and energy industries. However, inhalation of beryllium dust causes chronic beryllium disease (CBD), a granulomatous disease. Currently, to prevent occupational health problems, regulatory agencies have established a control concentration of 1-2 mg/m^3 in workplaces dealing with beryllium and beryllium compounds, but there is a report of the occurrence of CBD due to a much lower exposure concentration (0.02 mg /m^3) than the control concentration, and reinforcement of hygiene control against beryllium is sorely needed. Recent epidemiologic studies have indicated that compared to exposure to micro-sized beryllium dust particles, exposure to nano-sized beryllium dust particles contributes to the onset of CBD. Although this finding suggests that occupational health measures against nanoparticles are needed in workplaces dealing with beryllium, concrete measures focusing on beryllium nanoparticles to prevent CBD have never been discussed. In the future, it will be important to promote studies to assess the health effects of beryllium nanoparticles to prevent occupational health problems. To meet the needs of such a study, we focused on beryllium oxide nanoparticles and concentrated on inhalation exposure experiments using mice. We repeatedly examined the association between major histocompatibility antigen molecule (HLA-DPBI), which induces CBD, and beryllium oxide nanoparticles. Moreover, we established an atomization method using pulmonary surfactant solution and constructed an innovative inhalation exposure system that efficiently generates beryllium oxide nanoparticles into the air and allows the mice to inhale these nanoparticles.

Keywords: In vivo toxicology, Metal/metal oxide nanomaterials, Occupational safety

238. Biochemical Effects of Nano-TiO_2 with Different Surface Properties on Basil (Ocimum Basilicum)

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The current study is aim to investigate the role of surface modifications on the biochemical interaction of titanium dioxide engineered nanoparticles (nano-TiO_2, ENPs) with plants. In present study, basil (Ocimum basilicum) was cultivated for 65 days in soil amended with nano-TiO_2 with different surface properties (pristine, hydrophobic, or hydrophilic) at 0 (control), 125, 250, 500, and 750 mg·kg-1. Nanoparticles were characterized by traditional methods: transmittance electron microscopy (TEM) revealed three types of rutile nano-TiO_2 have a size of 25-70 nm. By using dynamic light scattering (DLS), pristine, hydrophobic, and hydrophilic nano-TiO_2 showed zeta potential of -14.5 ± 0.5 mV, 27.0 ± 0.9 mV, and 26.9 ± 0.5 mV, respectively. Titanium accumulation in plant tissues were determined by inductively coupled plasma-optical emission (ICP-OES). Relative chlorophyll content, carbohydrate concentration, and enzyme activities were determined by SPAD meter or UV/Vis spectrometry. Results showed a dose-dependent Ti accumulation in roots. However, Ti was not determined in shoots. With respect to control, hydrophilic nano-TiO_2 significantly decreased relative chlorophyll content. Pristine and hydrophobic nano-TiO_2 significantly suppressed ascorbate peroxidase (APOX) and catalase (CAT) activities in both roots and shoots, while hydrophilic nano-TiO_2 resulted a higher APOX activity in roots at 125 and 250 $mg \cdot kg^{-1}$. At 750 $mg \cdot kg^{-1}$, a comparison between three nano-TiO_2 showed that unmodified nano-TiO_2 significantly decreased reducing sugar concentration, and hydrophilic nano-TiO_2 reduced total sugar content while hydrophobic nano-TiO_2 revealed lower starch content. Results suggest that nano-TiO_2 with different surface chemistry could significantly affect biochemical parameters in basil plants, and potentially have unknown consequences for the food chain.

Keywords: Metal/metal oxide nanomaterials, TiO_2 nanoparticles, Surface chemistry, Basil, Carbohydrates, Antioxidant response

239. Identification of Quantum Dots with Reduced Toxicity for Human Health

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Quantum dots (QDs) are semiconductor nanocrystals with highly valuable optical and electrical properties for biomedical imaging as well as for optoelectronic devices. However, the most promising QDs, contain cadmium (Cd), which may induce adverse effects to health and the environment. Using a safer-by-design approach, our work aims at identifying QD with similar photoluminescence properties than Cd-based ones but exhibiting less toxicity throughout its life cycle, following artificial ageing in particular. In this work, the toxicity of Indium phosphide (InP) QDs, claimed to be a safer alternative, was evaluated in a human skin model (normal keratinocytes obtained from biopsies). Keratinocytes were exposed for 24 h either to hydrophilic InPZnS with ZnSe/ZnS double shell or InPZnS without shell. In addition, CdSe/ZnS was included as a positive control. Cellular internalization of QDs was observed by X-ray fluorescence microscopy and quantified by Inductively coupled plasma mass spectrometry (ICP-MS). InPZnS/ZnSe/ZnS QDs were not cytotoxic up to the highest tested dose (200 nM), as observed with the lactate dehydrogenase assay (LDH). However, the cell proliferation rate was dramatically reduced at 50, 100 and 200 nM compared to untreated cell. In addition, all the tested QDs induced DNA damage at 50 nM, as observed with the comet assay. These results suggest that nPZnSe/ZnSe/ZnS may be weekly toxic to human skin, although further experiments are required in order to elucidate the mechanism of toxic action. This work was carried out in the framework of Labex Serenade (ANR-11-LABX-0064) funded by the “Investissements d’Avenir” French Government program through A*MIDEX (ANR-11-IDEX-0001-02).

Keywords: Genotoxicity, Human toxicology, In vitro toxicology, Systems biology/toxicology

240. Three Months of Laser-Generated Nanoparticle Exposure Did Not Alter Cognition and Mood in Rats

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Laser ablation is a common material processing method in the industry and generates nanoparticles (NPs) as byproducts [1]. After their inhalation, nanoparticles reach the brain via both the olfactory neurons and the systemic circulation, which may lead to neurotoxicity and neuroinflammation [2]. It is shown that gold, silver and titanium nanoparticle exposure via different routes disrupt the spatial learning and memory functions [3]. We aimed to determine effects of nanoparticle inhalation during the material processing with laser ablation in an environment similar to that of industry on learning-memory and mood in behavioral, electrophysiological and molecular levels. Rats were exposed to copper, aluminum and tin NPs produced by ablation of plates using an industrial Nd:YAG laser for 2 hours/day for 90 days. The rats were subjected to behavioral tests and in vivo hippocampal field potential measurements at the end of 90 days. We did not observe significant changes in mood, learning and memory. The possible alteration of the N-methyl-D-aspartate (NMDA) receptor subunit expression in the hippocampus will also be analyzed. Our results imply that 3 months of exposure does not cause significant alterations in the brain. Since we have shown NP accumulation in the rat brain and humans are exposed to NPs for years, it is necessary to further examine the toxic effects of NPs by employing studies with longer exposure periods.

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Keywords: Exposure assessment, In vivo toxicology, Metal/metal oxide nanomaterials, Occupational safety

241. Exposure to a Mycobacterial Antigen, ESAT-6, Increases Fibrosis

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Recent studies suggest that environmental pollutants and microbial antigens may have additive effects on respiratory disease pathology. We established a murine model of environmental chronic granulomatous lung disease in which instilled multiwall carbon nanotubes (MWCNT) elicit granulomatous pathology with little evidence of fibrosis. Based on above studies, we hypothesized that the mycobacterial antigen, Early Secreted Antigenic Target Protein 6 (ESAT-6), a T cell activator associated with tuberculosis and granulomas, might have an adverse effect on pulmonary pathology. Wild-type C57Bl/6 mice received MWCNT with or without ESAT-6 peptide 14 [NNALQNLARTISEAG]. Controls received vehicle (surfactant-PBS) or ESAT-6 alone. Mice were evaluated 60 days later for pulmonary histopathology. Findings indicated that granulomatous changes and surprisingly fibrosis (as assayed by Trichrome staining and Fibronectin 1 expression) were markedly increased in mice receiving MWCNT + ESAT-6 compared to mice receiving MWCNT or vehicle alone. MWCNT + ESAT-6 also significantly increased T lymphocyte infiltration of lung tissues compared to MWCNT alone whereas ESAT-6 alone had no effect. Flow cytometric characterization of mediastinal lymph nodes revealed elevated Th2 and Th17 cells in mice instilled with MWCNT + ESAT-6 peptide but not with MWCNT alone. Findings suggest that ESAT-6 exacerbation of pulmonary disease in the MWCNT model involves expansion of pulmonary lymphocyte populations which promote fibrotic pathophysiology. These studies are important in improving our understanding of how underlying environmental pathology coupled with microbial insult may impact pulmonary disease outcome. Funding provided by NIEHS 022462 and North Carolina Biotechnology Center grant BRG12-06.

Keywords: Carbon-based nanomaterials, Environmental nanotoxicology, In vivo toxicology

242. Graphene Oxide Nanosheets Inhibition Cancer Cell Metastasis via Interaction with Actin Cytoskeleton

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Cytoskeleton is crucial in development and maintenance cell shape and cellular functions, which has been considered as an important drug target for cancer therapy. Recently, we reported that graphene oxide (GO) nanosheets can impair assembly of actin cytoskeleton, but its underlying molecular mechanisms and concomitant effects remain unknown. Here, we demonstrate that GO nanosheets can interact with cytoskeleton of cancer cells directly, which further alters cell functionality. Confocal fluorescence imaging experiments show GO nanosheets disturb actin cytoskeleton at a non-toxic concentration for cells. Circular dichroism spectrum assays further show GO nanosheets influence the secondary structure of actin. Additional cell studies reveal that the interaction between GO nanosheets with actin cytoskeleton decreases the metastasis abilities of cancer cells. Collectively, we uncover a novel cytotoxicity mechanism of graphene nanosheets and provide therapeutic opportunities for cancer therapy.This work is partially supported by the National Basic Research Program of China (973 Program Grant No. 2014CB931900), National Natural Science Foundation of China (21207164 and 31400862).

Keywords: Toxicological mechanisms, graphene, cytoskeleton, cancer, metastasis

243. Accumulation of Copper Oxide Nanoparticles in Gill, Liver and Muscle Tissues of Oreochromis Niloticus

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Accumulation of copper oxide nanoparticles (CuO NPs) in gill, liver and muscle tissues of Oreochromis niloticus was studied after exposing the fish to 20 µg/L Cu over 15 days. Experimental solutions were prepared using CuO nanopowder, (particle size <50nm) and metal levels in tissues were determined using an ICP-AES spectrophotometer. Statistical evaluation of the experimental data was carried out by a series of analysis of variance and t test. Copper is a basic element for the continuation of a number of metabolic functions such as hemoglobin synthesis, bone formation and it forms the structural components of enzymes. Exposure to this metal over certain concentrations, however, result in tissue accumulation and may alter various physiological functions. Nanoparticles (NPs) are defined as particles with dimensions between 1 - 100 nm and have unique properties such as high surface area due to their small size. Cu NPs are mainly used in electronic circuits, batteries, gas sensors and wood preservation. Production and use of engineered nanomaterials likely result in their release into aquatic environments and can lead to unexpected hazards on aquatic organisms. No mortality was observed during the experiments. Copper levels increased in gill and liver tissues of *O. niloticus* compared to control when exposed to CuO NPs whereas exposure to metal had no effect on muscle level at the end of the exposure period. Highest accumulation of copper was observed in liver while no accumulation was observed in muscle tissue. This might result from differences in metabolic activities of these tissues.

Keywords: Environmental nanotoxicology, Exposure assessment, Metal/metal oxide nanomaterials, Toxicological mechanisms, Copper oxide nanoparticles, Oreochromis niloticus, Accumulation

244. Genotoxicity of Cadmium Selenide Quantum Dots Evaluated Using Different In Vitro Assays

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Nanogenotoxicology is a field relevant to assessing potential genetic risk of nanomaterials. Quantum Dots (QDs) are widely used in biological applications, such as drug delivery, cancer diagnosis, fluorescent probes and biosensors. Conflicting data exist for QDs' toxicity and there is a lack of genotoxicity and mutagenicity data on CdSe QDs, which represents an important gap for QDs' risk-assessment. We selected two sizes of CdSe QDs (2.1-2.3 nm and 6.2-7.7 nm) to determine their mutagenicity and genotoxicity in mouse lymphoma cells in this study. The mouse lymphoma assay (MLA), Comet assay and micronucleus assay (MN) were conducted according to OECD guidelines to determine the mutations, DNA breaks, and chromosome damage induced by QDs. The QD size distribution, characterization in the culture medium, and cell uptake were determined before the treatment. The results showed that the cells could uptake the nanoparticles. The L5178Y/Tk+/--3.7.2C mouse lymphoma cells were treated with different doses of the QDs for the assays. The testing results showed that both sizes of the CdSe QDs were marginally mutagenic (2-fold increase in one or more doses) in the MLA, positive in the Comet Assay response with dose-dependently increased DNA breaks, and negative in the MN assay. The results suggest that CdSe QDs respond differentially to different genotoxic endpoints, most sensitive to the Come assay and less responsive to the MN assay. The possible underlying mechanism is being investigated for the differences.

Keywords: Genotoxicity, Cadmium Selenide Quantum Dots, Genotoxicity, in vitro toxicology

245. Poorly Soluble Co_3O_4P And $CoCl_2$ Exert Genotoxicity in Lung Cells by Different Mechanisms

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Cobalt is used in numerous industrial sectors, leading to occupational diseases, particularly by inhalation. Poorly soluble cobalt (II, III) oxide particles (Co_3O_4P) present an increased risk since they were shown to be retained into the lungs for long periods of time (years), enhancing thus their potential toxicity. Additionally, unsoluble Co_3O_4P are known to induce cytotoxicity via a Trojan-horse mechanism, which consists in a slow release of Co^{2+} following particles solubilization in the lysosomal compartment. Our study focused on the in vitro genotoxic potential of poorly soluble Co_3O_4P in BEAS-2B human bronchial cells, a cell line which exhibits the highest homology in gene expression pattern with primary nontumor cells and the lowest number of dysregulated genes compared to in vivo samples. While we did not observe cytotoxicity following exposure to poorly soluble Co_3O_4P, cytokinesis-block micronucleus assay and comet assay revealed that particles had a genotoxic potential. In fact, we observed enhanced micronuclei formation and both primary and oxidative DNA damage following exposure to increasing concentrations of Co_3O_4P. The scoring of γ-H2Ax foci demonstrated that Co_3O_4P were able to generate double DNA strand breaks, and the involvement of oxidative stress was confirmed by the inhibition of foci formation in the presence of the ROS scavenger N-acetylcysteine. Our study represents the first comprehensive genotoxicity study on poorly soluble Co_3O_4P particles. The comparison of the effects exerted by Co_3O_4P with those triggered by Co^{2+}, lead us to conclude that the genotoxic effects are independent of the amount of intracellular solubilized cobalt.

Keywords: Genotoxicity, In vitro toxicology, Occupational safety, Toxicological mechanisms

246. From Synthesis to Epi-Genotoxicity of Tritiated and Non Tritated ITER-like Tungsten Particles: the PASSIV-ITER project.

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Part of the material interacting with the plasma of the ITER thermonuclear fusion reactor (www.iter.org) will be made of tungsten that, for its mechanical properties and low plasma sputtering yield, guarantees reduced erosion of the tokamak inner wall during plasma operation, and therefore reduced particles production. Nevertheless, plasma-wall interaction can trigger the formation of tungsten nanoparticles (W-NP) that can be released in the environment, and cause occupational and accidental exposure. The assessment of the toxic potential of W-NP is thus of pivotal importance. We synthesized ITER-like W-NP by two different techniques (planetary ball milling and plasma), and we investigated their in vitro toxicological profile in BEAS-2B immortalized bronchial cells. The physico-chemical characterization of milled and plasma W-NP showed that both particles had a high specific surface area. Milled W-NP had the tendency to form aggregates, while the plasma-produced ones resulted more monodispersed.Analysis of the mitochondrial activity of BEAS-2B cells showed that W-NP exerted time- and concentration-dependent cytotoxicity, whereas cytostasis was not affected. Additionally, we investigated the genotoxic potential of W-NP by employing two techniques, the cytokinesis-block micronucleus cytome assay (CBMN-cyt) and the alkaline comet assay. CBMN-cyt showed that the frequency of micronuclei formation was concentration-dependent, as well as the primary DNA damage observed by comet assay. W-NP were additionally shown to induce significant oxidative stress in exposed cells.Effects of W-NPs on DNA methylation and histone modification are currently ongoing. Tritiated plasma W-NP will be additionally investigated, to better define the health risk in case of occupational exposure.

Keywords: Carcinogenicity, Genotoxicity, In vitro toxicology, Occupational safety

247. Effect of Airway Exposure to Carbon Black Nanoparticle on the Liver of Mouse Fed with N-3 Polyunsaturated Fatty Acids-Deficient Diet

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Previous studies have shown that exposure to particulate air pollutants exacerbates non-alcoholic fatty liver disease (NAFLD)-like phenotype in diabetic mouse and induces inflammation and triglyceride (TG) accumulation in the liver. Nanoparticle may exert systemic influence and hepatic lipid metabolism. The present study focused a potential role of n-3 polyunsaturated fatty acids (PUFAs), which is a natural antioxidants and regulates hepatic lipid metabolism, in nanoparticle toxicity, and was aimed to investigate the effect of exposure to carbon black nanoparticle (CB-NP) on mice fed with n-3 PUFAs deficient (N3def) diet. Six-week-old female C57BL/6J mice were fed control chow or N3def diet for 4 weeks to provide CTR and N3def groups, and then treated with CB-NP suspension or vehicle by intrapulmonary administration. Liver and blood samples were collected at 1 or 7 days post-treatment. While N3def diet intake increased hepatic expression of genes involved in fatty acid synthesis, CB-NP exposure further increased Scd1 expression level in the liver. CB-NP exposure and N3def diet intake synergistically increased lipid droplet accumulation in the liver. N3def diet intake may enhance hepatotoxicity induced by airway exposure to CB-NP.

Keywords: Carbon-based nanomaterials, In vivo toxicology, Toxicological mechanisms

248. Application of high content analysis for studying nanoparticles-cells interactions

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The use of nanomaterials (NMs) in industrial and consumer products continues to increase. To promote a safe and responsible application of NMs, it is fundamental to identify their potential adverse effects in biological systems and to connect them to the physicochemical properties of NMs. We have developed a multi parametric in vitro platform to assess the interactions of nanoparticles with cells using High-Content Analysis (HCA). A battery of assays that measures simultaneously changes in nuclear size and morphology, mitochondrial health, acidification of lysosomes, plasma membrane integrity and cellular morphology has been optimized. Moreover, we have applied HCA for the quantification of the uptake and intracellular localisation of fluorescent-labelled nanoparticles and to cross-link with omics findings in our group. Several examples of the potentiality of our platform will be presented. Results obtained show the great potential of this screening platform as a robust and reliable methodology for the safety evaluation of NMs. It allows assessing several toxicity mechanisms and cell events simultaneously, maximizing the experimental output while minimizing the input. Future prospects include broadening of the application to Nanomedicine and development of a screening platform to study drug efficacy and potential drug adverse effects.

Keywords: High throughput screening, In vitro toxicology, Physicochemical characterization, Toxicological mechanisms

249. Dissolution of Nanoparticles and the Dose at Target Sites: An Analysis of the Impact and Treatment of Dissolution in Dosimetry Models

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Dissolution of nanoparticles can expose cells to solubilised material and can affect particle size, transport and dose at the target site. We aim to evaluate the extent to which dissolution is considered in different in vitro and in vivo models and how it may affect the results obtained from these studies. The In vitro Sedimentation, Diffusion and Dosimetry (ISDD) model does not take into account dissolution within its configuration. On the other hand, the Volumetric Centrifugation Method (VCM) does consider dissolution of 'soluble' particles. The effective density from the VCM can therefore be utilized in the ISSD model to estimate the delivered dose. However, the VCM-ISDD does not provide mechanisms for modelling of polydisperse nanomaterials that undergo dissolution with time. On the other hand, the Distorted Grid (DG) Model has provisions for modelling of particles that undergo dissolution over time. The Agglomeration-Diffusion-Sedimentation-Reaction model (ADSRM) takes into account of the dissolution but requires detailed physicochemical data that may not be easily obtainable. Most in vivo dosimetry models are based on the International Commission on Radiological Protection (ICRP) model which was designed for poorly soluble particles. It can be shown that the dissolution has an impact on the target dose of many particles that are considered insoluble. Furthermore, dosimetry models have varying capabilities of dealing with dissolution. It can therefore be suggested that the dissolution kinetics of particles obtained from different biological media should be used to determine the choice of dosimetry model that need to be implemented.

Keywords: Dosimetry, In vitro toxicology, In vivo toxicology, Methods

250. A Novel Technique to Lyse Cryptosporidium Parvum using Silver Nanoparticles

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A novel technique is demonstrated to lyse the protozoan parasite, Cryptosporidium, enabling easier detection of this waterborne pathogen. The method uses silver nanoparticles to lyse Cryptosporidium. Silver nanoparticles (<20nm) are used to lyse enumerated samples of *Cryptosporidium* oocysts. The efficiency and confirmation of lysis are measured using a commercially available qPCR kit. The experiments were carried out from a spike level of 10000 oocysts down to 1 oocyst with signal observed at 10 oocysts. At 5 and 1 oocysts, signals received were not of sufficient confidence to be used. A silver nanoparticle concentration of 1mg/ml was used and the process demonstrated equal reaction efficiency to the traditional lysing technique of 10 repeat Freeze/Thaw cycles. At 50 spiked oocysts, the detection was of sufficient confidence for analysis. Silver nanoparticle lysis demonstrated a 96.65% correlation whilst Freeze/Thaw lysis demonstrated a 96.96% correlation for threshold cycle value vs logarithm of the spike levels. The effect of concentration of nanoparticles (1µg/ml to 10mg/ml) and exposure times (0 min to 2h) of silver nanoparticles was also explored. A spike level of 1000 oocyst was used for these experiments due to a good homogeneity observed in previous experiments. However, the results seem to provide no conclusive mechanism by which the nanoparticles interact with the cells. Interestingly, low silver nanoparticle concentrations and short exposure times showed equal effectiveness as high doses and longer times. A reaction efficiency close to 90% was observed with a slope of -3.098 for Freeze/Thaw and -3.47 for the Nanoparticles.

Keywords: Environmental nanotoxicology, Methods, Systems biology/toxicology

251. Nanoparticle Surface Properties Are a Key Descriptor for Cellular Uptake Kinetics

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When orally exposed, nanoparticles (NPs) can interact with the intestinal epithelial barrier leading to uptake and/or translocation of NPs. However, the influence of NP physicochemical properties on NP-cellular interactions are not fully understood. We systematically evaluated the effects of different physicochemical properties (two sizes and three surface functionalisations) using varying parameters (six doses at four time points). Subsequently, the influence of these properties and parameters on cellular uptake was mathematically modelled. Fluorescent polystyrene (PS) NPs (50 and 200 nm), functionalised with sulfone or carboxyl groups, at six doses (10-200 µg/ml) were exposed for 2, 3, 24, and 24h to Caco-2 intestinal cells. Confocal microscopy showed that all PSNPs accumulated in lysosomes, indicating a comparable uptake mechanism, and all studied parameters affected uptake. Cell-by-cell based high-content (HC) imaging analysis demonstrated NP uptake to be time and dose dependent until saturation occurred. For each PSNP the uptake rate (µg/ml/cell/hour) was calculated per dose and fitted using a linear regression model. Sulfone functionalisation resulted in the steepest slope (i.e. 0.18 with 35 µg/ml/cell/hour at 200 µg/ml), which was 36x higher than the shallowest slope, measured for one of the equally sized carboxyl functionalised PSNPs. Size comparison showed that uptake of 50nm particles was only 4x higher than that of 200 nm particles, indicating that surface chemistry outweighs the impact of size concerning NP uptake. Comparison of our data to literature shows similar trends between uptake and translocation data indicating that uptake studies based on HC screening could be a useful tool for bioavailability ranking studies.

Keywords: Biokinetics/toxicokinetics, Hazard ranking/characterization, High throughput screening, In vitro toxicology

252. Nanomaterial Shape-Dependent NLRP3 Inflammasome Activation

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The widespread and increasing use of engineered nanomaterials (ENM) increases the risk of human exposure, generating concern that ENM may provoke adverse health effects. In this respect their physicochemical characteristics are critical. The immune system may respond to ENM by inflammation. Inflammasomes are multiprotein complexes that assemble upon stimulation, resulting in activation of caspase-1 that in turn leads to production of interleukin (IL)-1β and IL-18, which are potent mediators of inflammation. The NLRP3 inflammasome is activated by a wide range of stimuli including ENM. Its activation is associated with various inflammatory diseases, including lung fibrosis, obesity and type 2 diabetes. We investigated effects of ENM shape on NLRP3 inflammasome activation. Two series of ENM were tested, one consisting of Ag and Au of different shapes (some of them PEGylated), and one of various chemical composition and shape. The ENM were extensively characterized, and tested for possible LPS contamination. PMA-activated THP-1 cells were used; IL-1b production and cell viability were measured. Ag 30x2 nm nanorods induced inflammasome activation, seen as dose-dependent increased IL-1b and concomitant reduced viability. Only one out of two similar (40x16 nm; 60x14 nm) PEGylated Au nanorods, showed inflammasome activation. Au 120x9 nm nanoprisms induced IL-1b with no effect on cell viability. CeO_2 12x3 nm stamps but not 4 nm spheres strongly reduced IL-1b and viability. In conclusion, our data may suggest shape-dependent inflammasome activation. ENM synthesis may involve surfactants; care should be taken that differences in response are not (partly) due to surfactant contamination. Supported by EU project FutureNanoNeeds (Grant agreement N°604602).

Keywords: Alternative testing methods/strategies, Emerging nanomaterials, Hazard ranking/characterization, In vitro toxicology, inflammation, inflammasome, shape, gold, silver, ceriumdioxide

253. Nanoparticles in the Lung - Progress and Barriers

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Background: The Comprehensive Toxicology book chapter on “Nanoparticles in the Lung” was published in 2011 and presented an integrated review of the known and hypothetical issues regarding medical, manufactured, and environmental nanoparticles. The chapter has been recently updated and this presentation will highlight the progress in the field over the past five years. Method: An extensive review of papers added to PubMed in the last five years was conducted. Findings: Hypotheses proposed previously continued to be studied with few ideas achieving consensus or being conclusively ruled out. It appears that a sufficient lung dose of any low solubility particle will have measurable effects, but there appears to be no sudden change in toxicological mechanisms in the size range between d>100 nm and 10 nm. Biodegradable nanoparticles with engineered characteristics continue to show promise for imaging and therapeutic purposes, but few applications have moved from research to clinical trials. Interpretation and Impact: Many early studies of nanoparticle toxicology were exploratory in nature, and a wide range of custom-synthesized particles were tested in cell culture or small animal models. Because of the diversity of particle types, biological models, and measured endpoints there are few cases where replicated results from multiple investigators have converged on generally accepted conclusions. Inclusion of shared reference materials as positive or negative controls in experimental designs is a possible way to allow integration of results across research groups.

Keywords: Environmental nanotoxicology, Human toxicology, In vitro toxicology, In vivo toxicology, Physicochemical characterization, Toxicological mechanisms

254. Assessment of Dermal Toxicity of Zinc Oxide Nanoparticles using Cultured Keratinocytes and a Human Epidermis Equivalent Model

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Assessment of skin irritation potential is fundamental on nanotechnology to know the potential risk for contact with skin in the manipulation process. Zinc oxide nanoparticles are currently being used for many industrial applications but little information is available on its skin irritation potential. This study was designed to determine the potential irritation of commercial zinc oxide nanoparticles of 50 and 100 nm compared to the non-nanometric zinc oxide, using cultured HaCaT keratinocytes and a human epidermis equivalent model. The different zinc oxides studied were incubated with the HaCaT cells for 24, 48 and 72 hours and were in contact with the human skin model for 24 hours. The viability of the cells was assessed in the two models by MTT method. Nanoparticles were characterized in different media (water, PBS and DMEM), determining their size by dynamic light scattering (DLS) and transmission electronic microcoscopy (TEM). Zinc oxide nanometric and non-nanometric particles reduced cell viability of HaCaT cells in a dose-dependent manner from 0.78 to 100 µg/mL. However, data obtained in skin equivalents revealed no irritation at a high concentration such as 1 mg/mLThis study indicates that zinc oxide nanoparticles do not cause acute cutaneous irritation independently of the size. The authors acknowledge financial support from the Ministerio de Economía y Competitividad Spain and European Union (FEDER) (Project MAT2012-38047-C02-01).

Keywords: In vitro toxicology

255. A Comparative Study of Catabolic Pathways Induced in Primary Macrophages by Pristine Single Walled Carbon Nanotubes and Pristine Graphene

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The correlation between the physico-chemical properties of carbonaceous nanomaterials and their impact on cells is critical to the risk assessment and safe translation into new engineered devices. Here the toxicity, uptake and catabolic response of primary human macrophages to pristine graphene (PG) and pristine single walled carbon nanotubes (pSWCNT) are explored, compared and contrasted. The toxicity was assessed using complementary techniques (live-dead assay, real time impedance technique and confocal microscopic analysis), which indicated no signs of acute cytotoxicity in response to PG or pSWCNT. TEM demonstrated that PG was phagocytosed by the cells into single membrane lysosomal vesicles, whereas primary macrophages exposed to pSWCNT contained double membrane vesicles indicative of an autophagic response. These discrete catabolic pathways were verified by biochemical and microscopic techniques. Raman mapping revealed that significant uptake and accumulation of the PG in discrete vesicles was recorded, in contrast to pSWCNT. Thermogravimetric analysis of the cells treated with PG revealed that 20-30% of the remaining dry mass was made up of PG.TEM analysis confirmed that PG was graphitic after 24 hours in the lysosomal compartments. In conclusion, these two types of nanomaterials with unique geometries differ significantly in their uptake mechanisms and induced lysosomal and autophagic catabolic pathways in human primary macrophages.

Keywords: Carbon-based nanomaterials, Emerging nanomaterials, In vivo toxicology, Toxicological mechanisms

256. Linking Nanomechanical Mechanisms to Carbon Nanotube-Induced Toxicity

Annette von dem Bussche, Wenpeng Zhu, Xin Yi, Yang Qiu, Zhongying Wang, Paula Weston, Robert Hurt, Huajian Gao, Agnes Kane. Brown University, USA.

The toxicity of carbon nanotubes has been widely studied, and due to variations in nanotube size, shape, dimensions, surface chemistry and impurities, investigations have shown different toxicity outcomes using various target cells in vitro. We hypothesize that nanomechanical interactions between one-dimensional nanomaterials and target cells are also important determinants of their toxicity. One-dimensional or high aspect ratio nanomaterials can mechanically damage biological structures including the plasma membrane, the cytoskeleton, and lysosomes. Following cell uptake, stiff or rigid one- dimensional carbon nanotubes may cause stress or deformation when packaged into soft spherical vesicles or lysosomes. We determine the precise material parameters that mechanically activate this toxicity pathway through coupled simulations and experimental studies using a panel of carbon nanomaterials with systematic variations in size, shape, and stiffness. Using biochemical assays and a variety of imaging methods in combination with quantitative single-cell confocal fluorescence imaging, we demonstrated that only long and stiff MWCNTs cause lysosomal permeabilization and release of cathepsin B, leading to activation of caspases and cell death. This study will contribute to a better understanding of nanomechanical mechanisms responsible for the toxicity of one-dimensional, high aspect ratio nanomaterials as a key to their safe design. This work was supported by NSF (CMMI-1028530, CBET-1132446 and CBET-1344097), Superfund Research Program NIEHS (Grant P42ES013660), and NICS (MS090046).

Keywords: Carbon-based nanomaterials

257. Cardiopulmonary Effects Of Inhaled Engineered Nanoparticles Released Across Life Cycle Of Nano-Enabled Products: The Case Study Of Nanoparticles Released From Laser Printers

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Numerous studies have investigated the possible adverse effects of inhalation exposure to engineered nanoparticles (ENPs). However, current exposures are not limited to pristine ENPs but include the particles released from nano-enabled products (NEPs) across their life cycle, named life cycle particulate matter (LCPM). To date, most studies have focused on the pulmonary effects of ENPs exposure, although epidemiological investigations have shown a strong association between inhalation of ambient ultrafine particles (diameter < 100 nm) and cardiovascular mortality and morbidity. There are limited studies on the cardiopulmonary toxicological potential of LCPM. In this study, a real-world LCPM test particle, engineered nanoparticles released during consumer use (laser printing) from nano-enabled toners (called PEPs) were used to expose male rats for 21 continuous days (5 hours/day) by whole-body inhalation. Throughout the exposure, real time instrumentation was used to monitor the exposure to both PEPs and gaseous pollutants. Further, the exposed rats were implanted with a telemetry system that allowed for the real time measurements of their electrocardiogram (ECG) and blood pressure fluctuations. Following the exposure, rats were sacrificed and bronchoalveolar and nasal lavage were performed. Analysis of the bronchoalveolar and nasal lavage fluid, as well as blood, was performed to assess for lung and cardiac injury and inflammation. Furthermore, gene expression signatures from exposed cells and animals will be screened to identify possible early biomarkers of cardiopulmonary dysfunction. Additionally, heart, lung, liver, spleen, kidney and tracheobronchial lymph nodes were harvested for histopathology assessment. The data thus far indicates that while there is absence of immediate lung injury, there is significant oxidative damage and inflammation following exposure to the pollutants emitted by the laser printer. Other endpoints are currently being measured to assess for cardiac injury and inflammation as well as changes in genetic expression. This study will allow for a more in depth understanding of the potential toxicological effect pollutants emitted from laser printers can have on the cardiopulmonary system. As such, this experimental design and exposure platform may also be used for evaluating the bioactivity of other LCPM relevant to current exposures.

Keywords: Environmental nanotoxicology, In vivo toxicology, Life cycle analysis

258. Low Toxicity of Dietary ZnO Nanoparticles to Young Mice

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As an important member of nanomaterial family, ZnO nanoparticles (NPs) have been produced in a large amount and extensively applied in different fields, including environment, cosmetics and food industry. These applications bring the exposure possibility of humans to ZnO NPs through oral administration. Since ZnO NPs have been added into food, especially infant and child food, more attention should be paid to the health risk of ZnO NPs to the young. Herein, the biodistribution and toxicity of ZnO NPs (20 nm) to the young mice were studied by feeding mice with the food supplementing ZnO NPs, submicro-sized ZnO (ZnO MPs, 500 nm) or zinc acetate for one and three months. Generally, our results indicated the low toxicity of the dietary zinc supplement. Compared with the young male mice, they induced more adverse damages in the young female mice. The dietary ZnO NPs were safer than ZnO MPs and Zn ions. In addition, ZnO NPs did not influence the gut bacterial phyla. After 90 days exposure to ZnO NPs, the two majority gut bacteria Bacteroidetes and Firmicutes phyla were not changed. The dietary zinc supplement did not increase the accumulation of zinc in organs except for the digestion tract organs. No significant difference among ZnO NPs, ZnO MPs and Zn ions was observed in bacterial phyla and distribution measurements. Contrary to many people's expectation, our findings suggest that ZnO NPs is safer than normal ZnO MPs when ZnO particles are added into food associated products for the young people.

Keywords: Biodistribution, In vivo toxicology, ZnO nanoparticle, feeding

259. Autophagy Dysfunction Contributes to the Hepatotoxicity of Silica Nanoparticles In Vitro and In Vivo

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Autophagy dysfunction is considered as an important toxic mechanism of nanomaterials recently. Our preliminary study found that silica nanoparticles induced autophagy and perhaps blocked autophagic degradation, while the specific mechanism remains unclear. Therefore, the present study is to explore the effect of silica nanoparticles on autophagy and autophagic flux in vitro and in vivo, and confirm the relationship between silica nanoparticles and autophagy dysfunction. Then explore the possible mechanism of autophagy dysfunction induced by silica nanoparticles. ROS inhibitor and gene-knockdown were used to explore that silica nanoparticles induce autophagy through ROS production and ER stress activation. To explore whether silica nanoparticles inhibited autophagic degradation is caused by lysosomal dysfunction, the pH change and the expression and enzyme activity of cathespin B and cathespin D in lysosome were measured. Therefore, this study clarified that silica nanoparticles promoted autophagosome formation and inhibited autophagosome degradation, leading to autophagy dysfunction. The present study provide theoretical basis for the toxic mechanism of silica nanoparticles, and have great scientific significance for the safety evaluation of silica nanoparticles.

Keywords: Emerging nanomaterials, silica nanoparticles,autophagy, endoplasmic reticulum stress, lysosomal dysfunction, molecular mechanism

260. Synchrotron Radiation Analytical Techniques in Studying Biomedical Effects of Metal Nanoparticles

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To understand nanotoxicity mechanism, it is crucial to capture in situ chemical information of nanomaterials (NMs) such as distribution and chemical forms, however, it remains a big challenge. It is necessary to use some in situ analytical methods to detect and characterize NMs in biological systems. Herein, we show some examples about using X-ray absorption near structure (XANES) to analyze binding structure of protein corona on NMs and the chemical speciation of metal NMs accumulated in cells, which is crucial for reveal chemical origins of cytotoxicity. We revealed that the binding structures of proteins on gold nanoparticles, which is via 12 Au-S bonds. The stably adsorbed protein corona plays important roles in reducing cytotoxicity as a natural shield to prevent NP surface to directly contact cell membrane. In addition, techniques like transmission X-ray microscope (TXM) and X-ray fluorescence microscope (XFM), and soft X-ray scanning and transmission microscope (STXM) are powerful to show in situ chemical identity of intracellular NMs with high resolution and sensitivity. The degradation and transformation of metal NMs seem to correlate to both nanotoxicity and their metabolism in vivo. With these techniques, we have clearly revealed the chemical origins of cytotoxicity for metallic NMs such as nanogold, nanosilver, and others. At last, we come to some prospects about the application of these novel techniques to study nanotoxicity in future. References:1. ACS nano, 2015, 9(6):6532-6547.2. Nanotoxicology, 2015, 9(2):181-1893. J. Am. Chem. Soc., 2013, 135(46):17359-17368This work is kindly supported by NSFC and Chinese Academy of Sciences.

Keywords: Biocorona, In vitro toxicology, Metal/metal oxide nanomaterials, Methods

261. Novel Analytical Methods used in Revealing Nanototoxicity Mechanism

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To understand the mechanism for nanotoxicity, it is crucial to capture in situ chemical information of nanomaterials (NMs) inside cells such as their distribution and chemical forms, however, it remains a big challenge. It is necessary to use some in situ analytical methods to image and characterize NMs in biological systems. Herein, we show some examples about the use of X-ray absorption near struture(XANES) to analyze adsorption struture of protein corona on NMs and the chemical speciation of metal NMs accumulated in the cells, which is crucial for reveal chemical origins of cytotoxicity.In addition, some X-ray based imaging techniques like transmission X-ray microscope(TXM) and X-ray fluorescence microscope(XFM), and soft X-ray scanning and transmission microscope(STXM) are powerful to show the in situ chemical identity of intracellular NMs with high resolution and sensitivity. With these techniques, we have clearly revealed the chemical origins of cytotoxicity for metallic NMs such as nanogold, nanosilver, and others. The stable protein adsorption or not and the degradation and transformation seem to initiate nanotoxicity. At last, we come to some prospects about frontier in the development and application of X-ray based techniques to study nanotoxicity in future. This work is kindly supported by NSFC and Chinese Academy of Sciences.

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Keywords: Biodistribution, Metal/metal oxide nanomaterials, Methods, Toxicological mechanisms

262. The Effect of Buoyancy on Nanoparticle Kinetics, Dosimetry and Cellular Toxicity

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The influence of nanoparticle (NP) kinetics is often disregarded in in vitro nanotoxicology and efficacy assessments. Yet, NP kinetics in culture media can significantly alter delivered dose metrics, hazard ranking, and nanomedicine efficacy. Here, we demonstrate that certain nanoparticles are buoyant in cellular studies due to low effective densities of formed agglomerates in culture media, which can reduce nanoparticle deposition, make it difficult to derive dose-response relationships and underestimate toxicity/bioactivity. The buoyancy phenomenon of NPs in-vitro is unknown in the fields of nanotoxicology and nanomedicine and has serious implications on how such NPs should be assessed in vitro. To investigate this issue, we determined the effective density of a test buoyant NP (polypropylene, PP) in culture media, performed colloidal characterization, assessed fate and transport of NPs in vitro using the Harvard DG computational model and developed an inverted 96-well cell culture platform in which adherent cells are incubated above buoyant particle suspensions to enable accurate dose-response assessment. Cellular responses to buoyant test NP were evaluated in human macrophages after 24 h incubation in both conventional and inverted systems. In conventional culture approach, no adverse effects were observed at any NP concentration tested (up to 250 μg ml^{-1}), whereas dose dependent decreases in viability and increases in reactive oxygen species were observed in the inverted system. This work elucidates the importance of nanoparticle kinetics and an unknown issue that plays a significant role on both in vitro hazard screening while providing a standardized methodology for cellular studies to assess buoyant NPs.

Keywords: Emerging nanomaterials, In vitro toxicology, Methods

263. Nanowaste: Toxicological implications of Released Particulate Matter from Incinerated Nano-Enabled Thermoplastics

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Nano-enabled thermoplastics are part of the growing market of nano-enabled products (NEPs) that have vast utility in several industries and consumer products. The use and disposal of NEPs at their end of life has raised concerns about the potential release of constituent engineered nanomaterials (ENMs) during thermal decomposition and their impact on environmental health and safety. One understudied nano-release scenario is incineration, where the thermo-decomposition of NEPs could release ENMs and gaseous pollutants of unknown toxicity. To investigate this issue, nano-enabled thermoplastics (polyurethane, polycarbonate, and ethylene vinyl acetate) containing carbon nanotubes (0.1% and 3% w/v) and titanium (2-15% w/v), respectively, along with the thermoplastic matrices were thermally decomposed using the Integrated Exposure Generation System (INEXS). The aerosolized particulate matter (LCPM) was monitored using real time instrumentation, size fractionated, sampled, extracted and prepared for toxicological analysis using human cell lines to assess potential nanofiller effects. Nanofiller effects were evaluated using common cytotoxicity and genotoxicity assays. The LCPM possessed an aerodynamic diameter of less than 100 nm and contained elemental carbon, polycyclic aromatic hydrocarbons (PAHs) and trace metals. We observed a nano-filler mediated toxic effect due to 24 h exposures of LCPM generated from thermally decomposed nano-enabled thermoplastics in comparison to the thermoplastic alone control. The enhanced bioactivity of certain LCPM derived from nano-enabled thermoplastics may be due to the presence of toxic four and five ring PAHs, which evolved from low molecular weight PAHs during incineration. This work highlights the importance of assessing LCPM and raises environmental health and safety concerns.

Keywords: Composite nanomaterials, Exposure characterization, In vivo toxicology, Life cycle analysis, Metal/metal oxide nanomaterials, Nanomaterial release

264. Nanoparticle-Microbiome Interactions - A New Area of Nanotoxicity?

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The human microbiome is defined as the sum of all microbial organisms that reside inside the body and include bacteria, fungi, and archaea. Recently, scientific and medical interest in the human microbiome has increased dramatically. In this respect, the type and the number of microorganisms as well as an impaired immune system play important roles in the outbreak of several diseases. Although, we are facing more than thousand reports on the impact of nanoparticles (NPs) and of the NP biomolecule corona on human cells, it is more than surprising that no studies on the relevance of NPs and the corona on the microbiome as well as on microbiome-host interactions have been reported thus far. Notably, different types of microorganisms are present in all the major exposure and entry sites for NPs in the human body as well as in environmental organisms. Therefore, we focused on the effects of NPs and the biomolecule corona on different types of microorganisms, as well as on cellular internalization of microorganisms in vitro. Additionally, we executed NP-microorganism internalization experiments in in vivo approaches, including light-sheet microscopy. The presented results provide novel insights in the impact of NP-microorganism interactions for humans and thus, may define a new area of nanotoxicology. Funding: BMBF, DENANA, NanoBEL, ChemBioMed.

Keywords: Biocorona, Microbiome, nanotoxicology

265. Comparison of Measured and Predicted Deposited Dose of Aerosolized Gold Nanoparticles in Rat and Mouse Lungs

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Predictive in silico models are used as tools for research and risk assessment to estimate deposition of inhaled materials throughout the respiratory tract. The Multiple Path Particle Dosimetry (MPPD) model for rats, humans, and mice (prototype) utilizes aerosol characteristics (count median diameter (CMD), mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), density) and physiological parameters (tidal volume, breathing frequency) to predict particle deposition fractions in the respiratory tract. Male Fisher 344 rats (2 months) and C57BL/6J mice (5 months) were exposed to spark-generated gold nanoparticle (NP) aerosols (CMD=20 nm, MMAD=302 nm, GSD=1.4) for 4 hours a day for 4 days. Lungs were harvested 24 hours post-exposure and deposited gold was measured by atomic absorption spectrometry. MPPD was used to estimate the daily deposited dose of gold in lungs given the same exposure conditions and physiological parameters as the in vivo model. The deposited dose in rat lungs (7.97±0.93 μg) was 1.5-fold lower than predicted using the MMAD and default physiological values (12.1 μg, minimal clearance expected). For mice, deposited dose was 11-fold higher than predicted (8.03±1.1 μg vs. 0.73 μg). Allometrically scaling the physiological values with the MMAD brings the predicted value closer to the actual deposited dose (6.84 μg vs 7.97 μg). The remaining differences could be explained by uncertainties in effective aerosol density. By comparing the predicted to actual deposited doses, we can better understand how aerosolized NPs behave.
Funding: NIH Grant R01ES020332, NIEHS Training Grant T32ES007026, NIEHS Center Grant ES001247

Keywords: Dosimetry, In silico modeling, Metal/metal oxide nanomaterials, Inhalation Exposure

266. A Toxicity Screening Platform Using Cryopreserved Precision Cut Lung Slices

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To mimic inhalation exposures to chemicals and engineered nanoparticles (ENP), immortalized cell lines are commonly used to assess biological responses. However, these cellular responses frequently differ from in vivo results, due to components of the native organ not present in cell-based systems. To more accurately reproduce organ complexity, precision cut lung slices (PCLS) have been adapted. Here, we evaluated the validity of cryopreserved murine PCLS for chemical toxicity testing by comparing them to never-frozen PCLS, commonly utilized human cell lines, and to living rat lungs. In response to the model toxin, $ZnCl_2$, we measured metabolic activity, mitochondrial membrane potential, total glutathione (GSH) levels and glutathione S transferase (GST) activity in never-frozen murine PCLS, frozen-thawed murine PCLS, human small airway epithelial and monocytic cells. In bronchoalveolar lavage fluids of $ZnCl_2$ instilled rats, we measured total and differential cell counts along with common markers of lung injury and inflammation. In cells and PCLS, exposure to $ZnCl_2$ induced a dose-dependent reduction in metabolic activity, mitochondrial polarization, and oxidative stress as measured by decreases in total GSH. Compared to never-frozen PCLS, frozen-thawed PCLS showed similar dose-response relationships for all measured endpoints. Finally, the rat lung lavage fluid also showed dose-related increases in indicators of cell membrane permeability and neutrophil degranulation, and reduction in GSH and GST activity. Frozen-thawed PCLS were more sensitive than in cell lines and were correlated with results in the whole animal in response to $ZnCl_2$. We envision cryopreserved PCLS as a useful platform for chemicals and potentially ENP-predictive lung toxicology.

Keywords: Alternative testing methods/strategies, precision cut lung slices, toxicology, zinc chloride, cryopreservation, high throughput screening, organotypic

267. Toxicological Consideration of Biomedical Nanomaterials

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Nanotechnology has shown great potential for the development of medical diagnosis, imaging and therapeutic agents for various diseases, includeing cancer. Various types of nanocarriers have been used for drug delivery, including organic ones (e.g. polymers, dendrimers, micelles, and liposomes) and inorganic ones (e.g. iron oxide, quantum dots, silver and gold nanoparticles). With the increasing applications of nanoparticles, research on their possible toxic effects has attracted considerable attention. Owing to the high surface area to volume ratio, nanoparticles may have distinct biological interactions compared to the bulk ones. The physicochemical properties (e.g. particle size, shape, surface coatings) of nanoparticle are considered as critical factors in controlling their pharmacokinetics, biodistribution, and biological effects. In this presentation, we will systematically report the effects of physicochemical characteristics on the cellular uptake, cytotoxicity, genotoxicity, reprotoxicity, and biodistribution of several nanoparticles, including iron oxide, quantum dots and gold nanoparticles.

Keywords: Biodistribution, Developmental nanotoxicology, Genotoxicity, Toxicological mechanisms

268. Intravenous Administration of Silver Nanoparticles Causes Organ Toxicity Through Intracellular ROS-Mediated Loss of Inter-Endothelial Junction

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Administration of AgNPs to mice could result in their distribution and accumulation in multiple organs, with notable prominence in liver, lungs, and kidneys. However, how AgNPs transport through blood vesicular system to reach the target organs is unclear, and the precise differences in the mechanisms of toxicity between AgNPs and silver ions still remain elusive. The aim of this work is to gain a new insight of AgNPs toxicity by comparing the mechanisms of action of AgNPs and $AgNO_3$. We investigated the in vitro cytotoxicity of either citrated-coated AgNPs or $AgNO_3$ upon primary HUVEC cells and the effects on intercellular conjunction and intracellular ROS. We also administered single or multiple intravenous injections to mice. Results showed that AgNPs were taken up by HUVECs. Meanwhile AgNPs induced the elevation of intracellular ROS and down-regulation of VE-cadherin between HUVECs. In contrast, $AgNO_3$ caused direct cell deathand without ROS induction at lower concentrations. We demonstrated that the internalization by HUVECs and intracellular ROS elevation was the initial step for AgNPs to disrupt the integrity of endothelial layer, which mediated peripheral inflammation in liver, kidney and lung. This work is not an official U.S. FDA guidance or policy statement. No official support or endorsement by the U.S. FDA is intended or should be inferred.

Keywords: Biokinetics/toxicokinetics, Exposure assessment, In vivo toxicology, silver nanoparticles

269. Effects of Elongated Silica Microparticles Enhanced Delivery of Tailorable Nanoemulsion as a Potential Platform for Transdermal Drug Delivery by NADH Signals from Coherent Anti-Stoke Raman Scattering (CARS) Microscopy

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The aim of our study is to use a lable-free, non-destructive imaging system, Coherent Anti-Stoke Raman Scattering (CARS) microscopy to investigate status of cells when the transdermal delivery of tailorable nanoemulsions (TNE) was enhanced by elongated microparticle (EMP) technology.The CARS approach here is specifically targeted to the lipid core of the TNE and nicotinamide adenine dinucleotide (NADH). We used TNE with a customized peptide surfactant, With the EMP-TNE technology, cutaneous delivery of a wide range of lipophilic payloads becomes possible. EMPs are not attached to any solid support allowing application to large areas of skin. Penetration of the stratum corneum by the microparticles creates pathways for the delivery of a wide range of bioactives. We have conducted preliminary experiments where EMPs were dry coated with TNEs. CARS imaging showed that the coating was uniform and stable for at least 72 hours. The dry form of EMP-TNE was applied to freshly excised human skin. Within 30 min, the EMP penetrated to the dermal-epidermal junction and we observed that the controlled release of TNE. TNE lipids could be detected as deep as 60 µm into the skin confirmed by CARS showing a potential usage of TNEs as a hydrophobic drug carrier. NADH positive signal was detected around EMPs in skin at the time of the delivery and attenuated after 30 min. In conclusion, EMPs could potentially provide enhanced delivery of TNEs, which leads to delivery of hydrophobic payload deep into the human epidermis and slowly release that payload in a controlled fashion.

Keywords: Biomedical/therapeutic applications, microparticles, nanoemulsions, transdermal drug delivery

270. Silica Nanoparticles Induce Macrophage Toxicity by Activating NLRP3 Inflammasome

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Objective: To investigate the mechanisms of inflammation caused by Silica nanoparticles. Method: The cell line of RAW 264.7 mouse macrophage were stochastically divided into five groups: negative control group, positive control group, 5 μg/ml administration group,10 μg/ml administration group as well as 20 μg/ml administration group. Morphological delineation was implemented 24h after incubation. The contents of SOD, CAT, LDH, GSH-Px and MDA in the supernates were determined to analyze the oxidative impairments of the cell. CCK-8 was exploited to assess the cytotoxicity of silica nanoparticles. Neutral red method was used to evaluate the phagocytic ability of macrophages. Western blot was carried out to detect NLRP3 inflammasome activation and the related pathways. Result: Compared to the negative control group, mcrophages in the silica nanoparticles-administrated groups manifested lower density and conspicuous aberrated morphological modifications. Oxidative damage tests demonstrated a lower enzyme activity of SOD, GSH-Px and CAT, a higher content of MDA and a higher enzyme activity of LDH. Meanwhile NLRP3, IL-1β and NF-κB were upregulated in positive control group and silica-administrated groups ($p < 0.05$) measured by Western blot. Conclusion: Silica nanoparticles could be toxic to macrophage cell by morphological structure change, engulfing ability damage. Additionally, silica nanoparticles can stimulate the upregulation of NF-κB pathway which will cause the activation of NLRP3 inflammasome to induce IL-1β secretion and inflammation.

Keywords: Exposure characterization, In vitro toxicology, Toxicological mechanisms

271. Influence of Graphene Oxide on the Growth, Structure and Function of White Rot Fungus Phanerochaete Chrysosporium

Sheng-Tao Yang, Jingru Xie. Southwest University for Nationalities, P.R. China.

Graphene and its derivatives have been widely investigated, produced and applied in diverse areas. Their toxicity to the environment should be fully considered to ensure the safe applications of graphene. Herein, we investigated the toxicity of graphene oxide (GO) to white rot fungus (Phanerochaete chrysosporium), which is important to the carbon and nitrogen cycling. The fresh and dry weights, pH, structure, ultrastructure, IR spectra and activity of the decomposition of pollutants were measured after 7 d exposure of P. chrysosporium to GO. Our results indicated that low concentrations of GO stimulated the growth of P. chrysosporium, while high concentration suppressed the growth. GO induced more acidic pH values of the culture medium and the disruption of the fibre structure of P. chrysosporium. The structural changes were reflected in the scanning electron microscopy investigations, where the disruption of fibres was observed. In the ultrastructural investigations, the shape of P. chrysosporium cells was changed and more vesicles were found upon the exposure to GO. The infrared spectroscopy analyses suggested that the chemical compositions of mycelia were not changed qualitatively. Beyond the toxicity, GO did not alter the activity of P. chrysosporium at low concentrations, but led to the complete loss of activity at high concentrations. These results collectively suggested that GO might induce hazard to white rot fungi and lead to ecological consequences. This work was financially supported by the China Natural Science Foundation (No. 201307101) and Top-notch Young Talents Program of China.

Keywords: Carbon-based nanomaterials, Environmental nanotoxicology, Toxicological mechanisms, white rot fungus

272. Neurotoxicity of Silica Nanoparticles: Brain Localization, Alzheimer's Disease-like Pathology and Spatial Memory Impairment

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Silica nanoparticles (SiNPs) are among the most produced nanomaterials and have been formulated for cellular and for non-viral gene delivery in the central nerve system. However, the potential toxic effects on brain remain poorly understood. Here, we found that SiNPs can enter the brain of rats treated with 15-nm SiNPs by intranasal instillation by transmission electron microcopy (TEM). SiNPs promote the release of pro-inflammatory cytokines and activate astrocyte cells in the brain. SiNPs decreases the total antioxidant capacity and increases the levels of malondialdehyde in the brain. In addition, SiNPs induce plaque-like AD pathology in the brain. ELISA analysis reveals that SiNPs significantly increase the production of β-amyloid 1-40/42 (Aβ1-40/1-42) with concurrent up-regulation of amyloid precursor protein. Western-blot analysis shows that phosphorylation of tau at Ser262, Ser396 and Ser404 is significantly increased, and that phosphorylated glycogen synthase kinase (GSK)-3β at Ser9 and phosphorylated catalytic subunit of protein phosphatase-2A (PP-2Ac) are significantly decreased in the brain. Activated GSK-3β and inactivated PP-2A may be responsible for increased phosphorylation of tau caused by SiNPs. Furthermore, SiNPs impair spatial memory of the rats but not learning abilities. In conclusion, our findings provide evidence demonstrating that SiNPs can enter the rat brains and that exposure to SiNPs induces neurotoxicity, AD-like pathology and memory impairment in the rats. These data suggest that application of SiNPs might cause neurotoxicity, increase the risk of developing AD pathology and cognitive deficit. Funding provided by Guangdong Provincial Natural Science Foundation (2014A030313715)

Keywords: In vivo toxicology, Exposure assessment, In vivo toxicology, Risk assessment, Toxicological mechanisms, Neurotoxicology

273. Interactions between Nanomaterials and Reactive Oxygen Species: Effects of Composition, Coating, Size, and Environment

Jun-Jie Yin, Wayne G Wamer. CFSAN/US FDA, USA.

The ability of nanomaterials to facilitate transfer of electrons and promote oxidative damage may be a fundamental property, arising from their small size and large surface to volume ratio. Assessments of nanomaterial safety must consider the possible toxicities arising from such properties. Using electron spin resonance (ESR), we studied two classes of nanomaterials with current or emerging commercial importance: noble metals (Ag, Au, Pt and Pd) and metal oxides (TiO_2, ZnO). Noble metal nanoparticles (NPs) demonstrate multiple enzyme-like (e.g. peroxidase, catalase, SOD) activities under different environmental conditions, suggesting that the bioactivity of noble metal nanoparticles may depend on the microenvironment pH in cells. While their catalytic activities appear similar, we observed pronounced differences among their mechanisms of catalysis. Studies on Ag and Pt NPs suggest potentially toxic interactions could occur between these NPs and antioxidant ingredients/components in foods, cosmetics, or dietary supplements. The reactivity of the metal oxides TiO_2 and ZnO were studied using ESR, biochemical, and cell-based methods. When excited by UV light, TiO_2 and ZnO, due to their semiconductor properties, can elicit toxicity through a free radical mechanism. We have demonstrated that depositing noble metal nanoparticles (Au, Ag, Pd NPs) onto a semiconductor (ZnO) is and effective way to enhance the photocatalytic and antibacterial activity of metal oxides. In addition, we have shown that ESR is a powerful tool for obtaining mechanistic information on the roles of ROS and holes/electrons in semiconductors' photocatalytic and antibacterial activities.

Keywords: Metal/metal oxide nanomaterials, Physicochemical characterization, Risk assessment, Toxicological mechanisms

274. Smart MoS_2 As an Effective Nanotheranostic for Cancer Imaging and Therapy

Wenyan Yin, Jie Yu. Institute of High EnergyPhysics, Chinese Academy of Science, P.R. China.

We reported a simple and high-throughput method for synthesis of MoS_2 nanoflakes via a modified oleum treatment exfoliation process [1]. By decorating with chitosan, these functionalized MoS_2 nanosheets have been developed as a chemotherapeutic drug nanocarrier for NIR photothermal-triggered drug delivery, facilitating the combination of chemotherapy and photothermal therapy into one system for cancer therapy. We further reported a multifunctional PEG-modified superparamagnetic MoS_2/Fe_3O_4 (MSIOs) nanocomposite with good biocompatibility obtained from a simple two-step hydrothermal route. The MSIOs was used as a novel theranostic agent for in vivo dual-modal MR and PAT imaging and the magnetic targeting MSIOs can more effectively ablate cancer. This nanocomposite has a great potential for MR/PAT imaging-guided focused magnetic targeting PTT of cancer spatially/timely controlled by the external magnetic field [2]. Consequently, a smart MoS_2 nanotheranostic has been investigated to realize the effective diagnostic and therapy of cancer. Due to its favorable NIR photothermal property combine with a versatile surface, the multifunctional anotheranostic would give more opportunities for future application in the biomedical fields. This work was supported by National Basic Research Programs of China (973 program, No. 2012CB932504 and 2015CB932104) and Beijing Natural Science Foundation (No.2162046).

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Keywords: Green nanomaterials, Nano MoS_2

275. Exposure Monitoring of Graphene Nanoplatelets Manufacturing Workplaces

Il Je Yu[1], Ji Hyun Lee[1], Jong Hun Han[1], Jae Hyun Kim[1], Boowook Kim[1], Dhimiter Bello[2], Jin Kwon Kim[1], Gun Ho Lee[1], Eun Kyung Sohn[1], Kyungmin Lee[1], Kyeongmin Lee[1], Kangho Ahn[1], Elaine Faustman[3]. [1]Institute of Nanoproduct Safety Research, Hoseo University, South Korea; [2]University of Massachusetts, Lowell; [3]University of Washington, USA.

This study investigates for the first time potential exposure of workers and research personnel to graphenes in two research facilities and evaluates the status of the control measures. One facility manufactures graphene using graphite exfoliation and CVD, while the other facility grows graphene on a copper plate using CVD, which is then transferred to a PET (polyethylene terephthalate) sheet. Graphene exposures and process emissions were investigated for three tasks - CVD growth, exfoliation, and transfer - using a multi-metric approach, using direct reading instruments, integrated sampling, and chemical and morphological analysis. Real-time instruments included a dust monitor, CPC, NSAM, SMPS, and an aethalometer. Graphenes and other nanostructures released from the work process were investigated using a TEM. Graphenes were quantified in airborne respirable samples as elemental carbon via thermo-optical analysis. The mass concentrations of total suspended particulate at workplace A and B were very low, and elemental carbon concentrations were mostly below the detection limit, indicating very low exposure to graphene or any other particles. The real-time monitoring, especially the aethalometer, showed a good response to the released black carbon, providing a signature of the graphene released during the opening of the CVD reactor at workplace A. The TEM samples obtained from workplace A and B showed graphene-like structures and aggregated/agglomerated carbon structures. Taken together, the current findings on common scenarios (exfoliation, CVD growth, and transfer), while not inclusive of all graphene manufacturing processes, indicate very minimal graphene or particle exposure at facilities manufacturing graphenes with good manufacturing practices.

Keywords: Carbon-based nanomaterials, Exposure assessment, Exposure characterization, Occupational safety, Carbon nanomaterials, Exposure assessment, exposure characterization, occupational safety

276. Subchronic Inhalation Toxicity Studies on Four Different Multiwalled Carbon Nanotubes

Il Je Yu, Jae Hyuck Sung, Byung Gui Choi, Kyung Seuk Song, Hyeon Yeol Ryu. Institute of Nanoproduct Safety Research, Hoseo University, South Korea.

Four different multiwalled carbon nanotubes (MWCNTs) were tested for 90 days subchronic inhalation toxicity along with acute and subacute tests. All the inhalation toxicity tests were conducted according to OECD test guidelines, TG 403, 412 and 413. All four MWCNTs were manufactured by chemical vapor deposit (CVD) method. In the subchronic inhalation toxicity tests, male and female rats were divided into four groups;), a fresh air control (0 mg/m^3), low (0.17 mg/m^3), middle (0.5 mg/m^3), and high (1 mg/m^3) concentration group. Each group consisted of 10 male and 10 female rats for 90 day exposure and 5 additional male rats for 90 day recovery after 90 day exposure. The rats were exposed to MWCNTs via nose-only inhalation 6 h per day, 5 days per week for 13 weeks. In addition to mortality and clinical observations, body weight and food consumption were recorded weekly. At the end of the study, the rats were subjected to a full necropsy, blood samples were collected for blood biochemical tests, and the organ weights were measured. No dose-dependent effects were recorded for the body weights, organ weights, bronchoalveolar lavage fluid inflammatory markers, and blood biochemical parameters at 1-day post-exposure and 90-day post-exposure. Histopathological examinations did not show any significant legions related to MWNCT exposure concentration. Four bundle type CVD manufactured MWCNTs with 15 nm diameter or less generated to dispersed tube structure aerosol for inhalation toxicity test did not show any significant pathological effects after 90 day inhalation exposure and 90 day post-exposure.

Keywords: Biodistribution, In vivo toxicology, Risk assessment, Carbon-based nanomaterials, in vivo toxicology, risk assessment

277. Reproductive toxicity of silver nanowire to Daphnia magna

Il Je Yu, Eun Kyung Sohn, Hyo Jin An, Hye Seon Park. Institute of Nanoproduct Safety Research, Hoseo University, South Korea.

Environmental exposure to nanoparticles can produce aquatic toxicity and be taken by aquatic species. Especially, Silver nanowire (AgNW) in aquatic media have unique properties such as surface charge, size distribution and aggregation/agglomeration. In order to understand chronic effect of silver nanomaterial into aquatic environment, the reproduction and molting of AgNW was assessed using D. magna reproduction test based on OECD test guideline 211 (Daphnia magna Reproduction Test). D. magna were exposed to AgNW for 21 days at concentrations 0.001, 0.005, 0.01, and 0.1 mg/liter. During the exposure period, medium and AgNW were replaced every 3 days. The total number of living offspring produced per parent animal alive at the end of test is assessed. The exposure to AgNW affected reproduction pattern of D. magna showing significant difference in the accumulative total offspring production while none of tested concentration caused the difference of molting number during 21days.

Keywords: Environmental nanotoxicology, In vivo toxicology, Metal/metal oxide nanomaterials, Environmental nanotoxicology, metal oxide nanomaterials, in vivo toxicology

278. Bioconcentration of Ag nanowire in Daphnia magna

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While the toxicities of AgNP have been known some extent, silver nanowires (AgNW) that are currently widely used in printed electronics and flexible display, are not clearly identified for their hazards. Furthermore, little is known about the ecotoxicity, accumulation and fate of AgNW in aquatic environments. This study proposes the accumulation of AgNW in Daphnia sp. as a crustacean, and the fate of AgNW in aquatic species. Two studies were conducted: 1) bioaccumulation of AgNW in Daphnia magna (D. magna) during 24 hr and 12 hr of depuration, 2) bioconcentration of AgNW including a 24 hr uptake period followed a 12 hr depuration to identify the accumulation profile of D. magna. As a result, Ag was found to be accumulated in the body of daphnia, and daphnia eliminates Ag from their body. This result showed a fast pattern of Ag depuration in D. magna to 6 hr after exposure during 24 hr depuration. Therefore, the high accumulation and fast depuration of AgNW correlate with the time to stay in gut of daphnia. Currently we are planning to identify chronic toxicity of accumulated AgNW.

Keywords: Environmental nanotoxicology, In vivo toxicology, Environmental nanotoxicology, Biokinetics, in vivo toxicology

279. Genes Induced in Response to Copper-Ion-Exposure and Differences of Gene Expression Between Copper-Ion and Nano Cuo in the Polychaeta Perinereis Aibuhitensis

Qianru Zhang. Institute of Applied Ecology, Chinese Academy of Sciences, P.R. China.

Ragworm (Polychaeta) is an ecologically important species in intertidal mudflats and estuaries and is recognized as a sential species for environmental monitoring. In the intertidal belt of China, the polychaeta Perinereis aibuhitensis is frequently exposed to various toxicants including heavy metals, organic chemicals, and emerging chemicals, such as nanomaterials, PPCPs, etc. However, little is known about the potential ecotoxicity of engineered nanoparticles (NPs) to them. To identify genes modulated by Cu^{2+}, two suppressive subtraction hybridization (SSH) cDNA libraries (forward and reverse) were constructed. A total of 239 differentially expressed clones were isolated and 87 of them were identified as Cu responsive. Reverse Northern results showed that 82 clones from forward library were up-regulated and 5 clones from reverse library were down-regulated in Cu-treated ragworms. Seventy-seven of them showed high homology to genes with known or putative function, and 10 were uncharacterized genes. Four full length cDNAs coding for a putative heat shock protein 70 (PaHSP70), a putative heat shock protein 90 (PaHSP90), a putative beta-1,4-endoglucanase (PaEG), and a putative matrix metalloproteinase (PaMT) were isolated and characterized. The expression levels of four genes shows different patterns under the different concentration of Cu^{2+} and nano CuO. Results suggest that the above four genes are likely to be involved in alleviation of Cu toxicity. The possible correlation between gene expression and Cu detoxication mechanism of ragworm, and differences of gene expression caused by Cu^{2+} and nano CuO are discussed.

Keywords: Exposure assessment, Genotoxicity, High throughput screening, Metal/metal oxide nanomaterials

280. Genotoxic Effects of Cobalt Nanoparticles: In vivo Studies

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Previous studies showed that exposure to cobalt nanoparticles (Nano-Co) caused oxidative stress and inflammation, which have been shown to be strongly associated with genotoxic and carcinogenic effects. However, few studies have been reported on Nano-Co-induced genotoxic effects in vivo. We propose that Nano-Co has genotoxic effects due to its high capacity of causing oxidative stress and inflammation. gpt delta transgenic mice were instilled intratracheally with Nano-Co (50 μg/mouse). After 7 days, extensive lung inflammation and increased cell proliferation which was reflected by increased Ki-67 and PCNA immunostaining in bronchiolar epithelial cells and hyperplastic type II pneumocytes were observed. Prominent positive γ-H2AX immunostaining was observed in the nuclei of bronchiolar and alveolar epithelial cells in the lungs exposure to Nano-Co. After four months, extensive interstitial fibrosis and proliferation of interstitial cells with inflammatory cells infiltrating the alveolar septa were observed. Increased nuclear Ki-67 and PCNA immunostaining was also observed in some bronchiolar epithelial cells and bronchiolized cells. Moreover, exposure of mice to Nano-Co caused increased level of 8-OHdG in genomic DNA from mouse lung tissues. These results suggest that intratracheal exposure of mice to Nano-Co resulted in oxidative stress and lung inflammation and cell proliferation. The mutant frequency and mutation spectrum in gpt gene were also determined in mouse lungs four months after Nano-Co exposure. Our results showed that Nano-Co induced a much higher mutant frequency as compared to the controls, and the most common mutation was G:C to T:A transversion, which may be explained by Nano-Co-induced increased formation of 8-OHdG.

Keywords: Genotoxicity, Cobalt nanoparticles, DNA damage, 8-Oxo-2'-deoxyguanosine, histone H2AX phosphorylation, mutation, Oxidative stress, titanium dioxide nanoparticles

281. Inhibition of Human Mesenchymal Stem Cell Adipogenic Differentiation by Titanium Dioxide Nanoparticles

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Titanium dioxide (TiO_2) has been widely used in the consumer products, including food, cosmetics and various medical products. While many studies focused on the biomedical application of TiO_2, little is known about the impact of nanoscale TiO_2 on human stem cells. In this study, we investigated the effect of TiO_2 nanoparticles (NPs) on adipogenic differentiation of human mesenchymal stem cells (hMSCs). Several different sizes of TiO_2 NPs both in rutile and anatase phase were investigated. Physico-chemical characterization of TiO_2 NPs was first conducted using electron microscopy (EM), x-ray diffraction and dynamic light scattering techniques. Subsequently, cytotoxicity of TiO_2 NPs was evaluated using LDH, ATP assays, and the adipogenic differentiation capacity was assayed by Oil red Staining. Interestingly, TiO_2 NPs exhibited minimal short-term cytotoxicity in hMSCs; however, the size- and crystal structure- dependent inhibition of hMSC adipogenic differentiation by TiO_2 nanoparticles was noted. Furthermore, media "stripping" studies indicated that the inhibition of hMSC adipogenesis was likely due to direct cellular response to TiO_2 NPs instead of the "charcoal" effect. Cellular uptake of NPs was evaluated by EM; all tested NPs were localized in the cytoplasm of hMSCs. Moreover, the PCR array analysis suggested that TiO_2 NPs inhibited hMSC adipogenesis by down-regulating key genes involved in adipogenesis promotion, including FGF2, IRS1, CEBPA, CEBPB, and ACACB. In conclusion, nanoscale TiO_2 inhibited hMSC adipogenic differentiation, and size and crystal structure of the particles play important roles. This research is supported by FDA Commissioner's Fellowship Program and NCTR-ORA Nanotechnology Core Facility.

Keywords: In vitro toxicology, Metal/metal oxide nanomaterials, Titanium dioxide, Stem cells

282. Sensing Solution for Airborne Carbon Nanotube Exposure in Workplaces Based on Surface Enhanced Raman Spectroscopy

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Today's advances in man-made nanomaterials pose new and unprecedented health risks, arising especially from airborne, inhalable fiber-shaped nanomaterials, like carbon nanotubes (CNTs). In vivo studies indicate that inhalation of CNTs can cause adverse pulmonary effects including inflammation, granulomas and pulmonary fibrosis [1, 2]. As a result, the National Institute of Occupational Health and Safety (NIOSH) recommends an exposure limit of 1ug/m3 of CNTs as a respirable mass 8-hour time-weighted average concentration [3]. However, detecting this amount is extremely challenging with the current sensing solutions. Here, we would like to present a wearable, cost-effective badge sensor with an air filtration system [4,5]. The sensor is capable of collecting airborne carbon nanotubes from the surrounding atmosphere on a disposable membrane filter, which acts as a SERS substrate. The badge system is integrated with a bench-top sized optical reader for fast and automated inspection of collected samples. Our system enables detection of sub-nanogram quantities of collected CNTs and, by utilizing the advantages of Raman spectroscopy, is a solution able to uniquely distinguish carbon nanotubes from background aerosols present in air.

[1] Nat Nanotechnol. 4(11): 747–751 (2009); [2] Am J Physiol Lung Cell Mol Physiol 289: 698-708 (2005); [3] NIOSH CIB 65: Carbon Nanotubes and Nanofibers; [4] Patent pending ; [5] Swiss Technology and Innovation Project 17623

Keywords: Exposure assessment, Exposure characterization, CNT, sensor

283. Genotoxicity Assessment of Silica and Titania on BEAS-2B Cells in the Framework of EU Nanoreg Project

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Genotoxicity assessment is an important aspects in establishing nanoparticle (NP) safety. In the framework of NaNoReg project, genotoxicity will be evaluated by different approaches including the CBMN assay. Results will support the decision tree and the regulatory framework/toolbox, two of the main outputs of the project. In this context, we applied the CBMN assay on Beas-2B cells, a human lung cell model to test four NPs, two silica (NM200 and NM203) having different surface properties and two Titania (NM100 and NM 101) having different size. Primary efforts were devoted to uniform among all partners, the NP dispersion protocol according to the NaNoREG refined Nanogenotox dispersion protocol based on MilliQ-BSA (0.05%) suspension and probe sonication. Characterization has been performed by DLS on batch suspension as well as on NM suspension in media at the beginning and at the end of the treatment. The CBMN assay has been performed according to the shared protocol and to OECD TG 487. Beas-2B were treated for 48 h with NP concentrations from 0.1 to 100 µg/ml. Cyt B was added 6 hours after the beginning of treatment. DLS characterization indicated a good reproducibility of Z-average and PDI of batch dispersion and great variability after dispersion in cell medium both at the beginning and at the end of the treatment. In the CBMN assay, replication index indicated greater toxicity of NM203 than NM200 possibly due to surface differences not relevant for micronucleus induction. This study is partially financed by FP7 NANoREG project, Grant n. 310584.

Keywords: Genotoxicity, Human toxicology, In vitro toxicology, Toxicological mechanisms

284. Metabolomic effects in HepG2 cells exposed to CeO_2, SiO_2 and CuO nanomaterials

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To better assess potential hepatotoxicity of nanomaterials, human liver HepG2 cells were exposed for three days to 5 different CeO_2 (either 30 or 100 ug/ml), 3 SiO_2 based (30 ug/ml) or 1 CuO (3 ug/ml) nanomaterials with dry primary particle sizes ranging from 15 to 213 nm. Metabolomic assessment of exposed cells was then performed using four mass spectroscopy dependent platforms (LC and GC), finding 344 biochemicals. Four CeO_2, 1 SiO_2 and 1 CuO nanomaterials increased hepatocyte concentrations of many lipids, particularly free fatty acids and monoacylglycerols but only CuO elevated lysolipids and sphingolipids. Observed decreases in UDP-glucuronate (by CeO_2) and S-adenosylmethionine (by CeO_2 and CuO) and increased S-adenosylhomocysteine (by CuO and some CeO_2) suggest that a nanomaterial exposure increases transmethylation reactions and depletes hepatic methylation and glucuronidation capacity. There was a clear pattern of nanomaterial-induced decreased nucleotide concentrations coupled with increased concentrations of nucleic acid degradation products. An earlier observation of CeO_2 induced lipid elevations has been confirmed by 4 other different CeO_2 as well as SiO_2 and CuO nanomaterials. Additionally some metal oxide nanomaterials induced declines in S-adenosylmethionine, UDP-glucuronate, dipeptides, 6-phosphogluconate, NADPH and NADH. Disclaimer: This abstract does not necessarily reflect EPA policy.

Keywords: cerium oxide nanoparticles, metal oxide nanoparticles

Abstracts for oral presentations

1. Effect of pH-Varied Cerium Oxide Nanoparticles on the Growth of Gram-Positive and Negative Bacteria

Ece Alpaslan, Thomas J. Webster. Northeastern University, USA.

Cerium oxide nanoparticles (nanoceria) have been used in variety of applications including biomedical applications. The oxygen defect structure on its surface and alternating oxidation state between +3 to +4 gives ceria a unique property of modulating reactive oxygen species (ROS) levels, which allows for their use as a therapeutic agent to fight against cancer and many other ROS associated diseases. Despite the promise of nanoceria as a therapeutic agent for cancer applications, it has not been extensively studied for antibacterial activity. In the current study, the antibacterial activity of dextran-coated nanoceria was examined against Pseudomonas aeruginosa (as a gram-negative bacteria) and Staphylococcus epidermidis (as a gram-positive bacteria) in terms of a dose, time and pH dependent manner. A non-linear growth equation (Gompertz Equation) was fitted to the experimentally collected data and parameters associated with bacteria growth as maximum specific growth rate (μ), lag time (λ), and the total amount of bacteria (A) were calculated. Findings suggest that dextran-coated nanoceria particles were much more effective at killing P. aeruginosa and S. epidermidis at basic pH (pH=9) compared to acidic pH values (pH=6). Between different bacteria strains at pH 9,P. aeruginosa growth was delayed for few hours between different particle concentrations, whereas S. epidermidis did not grow when treated at 500 μg/mL nanoceria concentrations for 24 hours. This study provides significant evidence for the use of nanoceria for a wide range of anti-infection applications without resorting to the use of antibiotics.

Keywords: Cerium oxide nanoparticles, antibacterial properties, Oral

2. Material Deposition in the Respiratory Tracts of Humans and Animals

Bahman Asgharian. Applied Research Associates, Inc., USA.

Assessment of the dose and site of deposition of inhaled materials in the lung aids in the interpretation of biological response. The deposited dose provides the link between external exposure and potential adverse effects. While high fidelity computations of the dose are only feasible for the proximal airways of the lung which fall within the resolution of scanned images, anatomically accurate and physiologically relevant mathematical models of material transport and deposition in the entire respiratory tract can be developed for humans and selected animals to predict site-specific dose and tissue uptake, and allow interspecies extrapolation based on various dose metrics. Mathematical models have been formulated for shape particles such as nanoparticles. Modeling approach and application to several scenarios will be discussed and a few examples will be presented.

Keywords: Biodistribution, Dosimetry, In silico modeling.

3. Using Toxicokinetic Modeling to Describe and Predict the Fate of Inorganic Nanoparticles in the Body

Gerald Bachler. Shell International, Netherlands.

Background: During the last couple of years extensive research was conducted to investigate the toxicokinetics (TKs) of inorganic nanoparticles (NPs). The interpretation of the generated data is, however, hampered by the fact that numerous factors influence the fate of NPs in the body (e.g. size, coating, dose, agglomeration, etc.). TK Modeling: An efficient method to aggregate existing experimental TK data is physiologically based pharmacokinetic (PBPK) modeling. Recently, the first PBPK models were introduced that have successfully been used to (1) describe the TKs of NPs on the bases of key physiological parameters (i.e. translocation through the capillary wall and uptake by the macrophage system), (2) predict the results of in vivo experiments obtained with a wide variety of different particle types and exposure conditions (i.e. chemical composition, size, coating, exposure route, species), (3) describe the dissolution kinetics of silver NPs in vivo and de novo-formation of secondary particles, and (4) establish a link between the translocation of gold NPs across alveolar epithelial cellular monolayers with data obtained by animal inhalation experiments. Conclusion: TK modeling is a powerful method that greatly supports the interpretation of in vivo data, can indicate the design of future in vivo studies, and enables the identification of key physiological parameters that determine the biodistribution of NPs. Thus, having the potential to reduce animal studies and facilitate interspecies extrapolation. Funding provided by the German Federal Institute for Risk Assessment (BfR), the Swiss Federal Office of Public Health (FOPH) and the Adolphe Merkle Foundation.

Keywords: Biodistribution, Biokinetics/toxicokinetics, In silico modeling, Metal/metal oxide nanomaterials.

Oral

4. In Vitro Toxicity of Nanoceria: Effect of Coating and Stability in Biofluids

Jean-François Berret, Nawel Ould-Moussa, Malak Safi, Marie-Alice Guedeau-Boudeville, H. Conjeaud, Hélène Conjeaud. Laboratoire Matière et Systèmes Complexes UMR 7057 Université Paris-Diderot/CNRS, France.

Due to the increasing use of nanometric cerium oxide in applications, concerns about the toxicity of these particles have been raised and have resulted in a large number of investigations. We report here on the interactions between 7 nm anionically charged cerium oxide particles and living mammalian cells. By a modification of the particle coating including low-molecular weight ligands and polymers, two generic behaviors are compared: particles coated with citrate ions that precipitate in biofluids and particles coated with poly(acrylic acid) that are stable and remain nanometric [1,2]. We find that nanoceria covered with both coating agents are taken up by mouse fibroblasts and localized into membrane-bound compartments. However, flow cytometry and electron microscopy reveal that as a result of their precipitation, citrate-coated particles interact more strongly with cells. At cerium concentration above 1 mM, only citrate-coated nanoceria (and not particles coated with poly(acrylic acid)) display toxicity and moderate genotoxicity. The results demonstrate that the control of the surface chemistry of the particles and its ability to prevent aggregation can affect the toxicity of nanomaterials [3].

[1] B. Chanteau et al., Langmuir 2009, 25(16), 9064 – 9070; [2] M. Safi et al., Nanotechnology

21, 145103 (2010); [3] N. Ould-Moussa et al., Nanotoxicology 8, 799 – 811 (2014).

Keywords: Biocorona, Cerium oxide nanoparticles, In vitro toxicology, Physicochemical characterization

5. Prenatal Nephrotoxicity and Maternal Nanomaterial Inhalation

Jason Blum, Judith T. Zelikoff. Department of Environmental Medicine, New York University School of Medicine, USA.

The effects of nanoparticle (NP) exposure during pregnancy on the fetus and neonate are poorly studied and thus poorly understood. For these studies, timed-pregnant CD1 mice were exposed by inhalation throughout gestation to nanosized cadmium oxide (CdO), an occupationally and medically-relevant metal oxide. Effects of maternal exposure to CdO were examined on: dam and offspring body and organ weights; obstetric parameters, including birth weight, gestational duration and birth length; and fetal and neonatal growth. Results demonstrated that CdO nanoparticles inhaled during pregnancy translocated from the local site of deposition (i.e., lungs) to a variety of distant organ systems including the placenta, uterus and mammary glands. In addition, CdO exposure decreased fecundity and neonatal growth, while altering placental weight in a time-dependent manner. In addition, nephrotoxicity was observed in both the dams and neonatal offspring as determined by time-dependent increased expression of kidney injury marker (KIM)-1 using RT PCR; while NGAL expression in the kidney was increased in the dam, similar effects were not observed in the neonates. As commercial uses for nanotechnology continue to expand throughout the world, risks for unintentional exposure in the workplace and intentional exposure associated with medical strategies, increase. This presentation demonstrates the public health implications for pregnant women and their unborn children associated with maternal inhalation during pregnancy of certain metal oxide nanoparticles.

Keywords: Developmental nanotoxicology, Metal/metal oxide nanomaterials

6. Susceptibility to ENMs in Mouse Models of Allergen-induced Lung Disease

James Bonner. North Carolina State University, USA.

Engineered nanomaterials (ENMs), including carbon nanotubes (CNTs) and nanometals, pose risk for susceptible individuals with pre-existing lung diseases such as asthma due to adjuvant-like properties. Alternatively, some ENMs have the potential to cause allergic lung disease directly through polarization of T-helper cells towards a Th2 phenotype. While there is no epidemiological evidence to showing that ENMs cause or exacerbate lung diseases in humans, there is compelling evidence from rodent studies demonstrating that ENMs stimulate immune and allergic adjuvant-like responses in the lung (e.g. eosinophilic inflammation, mucous cell metaplasia, airway fibrosis), suggesting a possible risk for allergic disease in humans as a consequence of occupational, environmental, or consumer exposure. We have shown that pre-existing allergic lung inflammation in mice increases chronic airway remodeling in response to CNTs or metal nanoparticles delivered by inhalation exposure or by bolus dose delivered via oropharyngeal aspiration. The mechanisms mediating the exaggerated allergic responses to these ENMs during pre-existing lung inflammation involve increased production of soluble cytokines and growth factors (e.g., PDGF, CCL2, TGF-b1), suppression of inflammasome activation and IL-1b release, and enhanced responsiveness of lung connective tissue cells (fibroblasts) through altered expression of cell-surface cytokine receptors or dysregulated intracellular signaling patterns. Moreover, we have also identified specific transcription factors (e.g., STAT1, Tbet) that mediate genetic susceptibility to ENM-induced lung disease in mice. Collectively, these studies contribute to nanosafety research through identifying mechanisms of susceptibility to ENMs that should allow for the safe design of ENMs and the prevention of future allergic lung disease.

Keywords: In vivo toxicology, Toxicological mechanisms

7. Long-term Impact and Biodegradation Kinetics of Chemically Functionalized Carbon Nanotubes within Primary Microglia

Cyrill Bussy[1], Alberto Bianco[2], Maurizio Prato[3], Kostas Kostarelos[1]. [1]University of Manchester, UK; [2]CNRS, France; [3]University of Trieste, Italy.

Chemically functionalized carbon nanotubes (f-CNTs) are promising nanovectors for brain applications. When interacting with the brain parenchyma, they are shown to encounter microglial cells. Our previous short-term in vitro studies suggested that microglia have a key role in the toxicity of f-CNTs to glial cells in a dose dependent manner, but irrespective of the surface functionalisation of the CNTs. Here, we questioned whether the type of surface functionalization could modulate the biological response and physiological functions of microglia when exposed to a non-toxic dose over a long period of time (1 month). Inflammogenicity of different f-CNTs (carboxylation, amination, and combined carboxylation and amination) and their interference with three major microglia functions, namely phagocytosis, degradation and migration were investigated. Isolated primary microglial cells showed good over time viability following exposure to and uptake of materials regardless of the f-CNTs characteristics. In addition, no sign of inflammation or itrosative stress were found. Similarly, phagocytosis and degradation abilities were not affected. For all 3 materials, structural alteration of the intracellularly-residing nanotubes suggested partial but continuous degradation over time. Noticeably, biodegradation was found more pronounced for carboxylated materials. Finally, f-CNT-loaded microglia were still able to migrate irrespective of the type of material they internalized. However, a higher number of microglia exposed to carboxylated CNTs were migrating upon stimulation in comparison to aminated ones. Overall, this data showed that the primary microglia functions are not altered by exposure to f-CNTs, but that variations in surface modification of the material is key to control biological responses.

Keywords: Biokinetics/toxicokinetics, Biomedical/therapeutic applications, Carbon-based nanomaterials, In vitro toxicology

8. Time-dependent Changes in Brain Induced by Intravenously Delivered Cerium Oxide Nanoparticles Are Consistent with Potential Pathways Discerned by Proteomics Identification of In Vivo Serum or Plasma Proteins Adsorbed to Nanoceria

D. Allan Butterfield[1], Rukhsana Sultana[1], Jason Unrine[1], Uschi Graham[1], Eric A. Grulke[1], Michael T. Tseng[2], Jon B. Klein[2], Robert A. Yokel[1]. [1]University of Kentucky; [2]University of Louisville, USA.

Nanoceria are proposed as therapeutics for numerous disorders. We confirmed up to 30 days following i.v. nanoceria addition to naïve rats time-dependent effects in brain were similar to those previously observed in nanoceria-treated cell cultures in vitro and the results were fit into an extant, 3-tier hierarchical oxidative stress model. However, longer times (90 days) post-treatment led to normalization of brain oxidative stress and neuroinflammation. This time coincided with bioprocessing of nanoceria in the liver to produce ultra-fine nanoparticles with more antioxidant Ce(3+) as confirmed with high resolution imaging and electron energy loss spectroscopy, decreased oxidative stress and decreased neuroinflammation, thereby for the first time extending this hierarchical oxidative stress model to a fourth tier. To gain more insights into these changes, proteomics identifications of plasma/serum proteins adsorbed to nanoceria in vivo were conducted. The identified proteins regulate numerous cell functions and are consistent with our observations in brain of nanoceria-treated rats. Additional studies are ongoing in our laboratory to better understand the role of the plasma/serum protein corona on nanoceria's effects on brain and other organs. Funding provided by EPA RD-833772; R01GM109195.

Keywords: Biocorona, Cerium oxide nanoparticles

9. Proteomics Identification of In Vivo Serum or Plasma Proteins Adsorbed to Nanoceria: Insights into Potential Pathways by which Cerium Oxide Nanoparticles May Exert Beneficial or Deleterious Effects

D. Allan Butterfield[1], Rukhsana Sultana[1], Jason Unrine[1], Uschi Graham[1], Eric Grulke[1], Michael Tseng[2], Jian Cai[2], Jon B. Klein[2], Robert A. Yokel[1]. [1]University of Kentucky; [2]University of Louisville, USA.

Nanoceria are proposed as therapeutics for numerous disorders. Systemic or pulmonary administration is required due to limited oral absorption. In blood, nanoceria becomes protein-coated (opsonized), changing its surface properties, yielding different cellular presentations.Nanoceria-blood protein interactions information and role of the protein corona on nanoceria functions is lacking. Serum/plasma-bound proteins could influence nanoceria's biological properties and relate to conflicting literature on its protective vs. harmful effects. Consequently, identifying adsorbed blood proteins on nanoceria is critical. This study is the first proteomics identification of plasma/serum proteins adsorbed to nanoceria in vivo. The identified proteins regulate numerous cell functions: antioxidant/detoxification, energy regulation, signaling, immune, iron homeostasis, proteolysis, inflammation, among others. The implications are: 1) Specific protein coronas may affect nanoceria uptake and subsequent organ bioprocessing; 2) nanoceria-adsorbed proteins may have altered structure, inducing altered functions and/or changed nanoceria function. Consequently, prior to use as therapeutic agents, better understanding of behavior of opsonized nanoceria on cellular/organ function is required, studies ongoing in our laboratory. Funding provided by EPA RD-833772; R01GM109195.

Keywords: Cerium oxide nanoparticles, Proteomics; Nanoceria blood protein corona

10. Physiologically Based Pharmacokinetic Modeling of Cerium Dioxide Nanoparticles Infused Intravenously into Rats

Ulrika Carlander[1], Gunnar Johanson[1], Anteneh Assefa Desalegn[1], Robert Yokel[2]. [1]Karolinska Institutet, Sweden; [2]University of Kentucky, USA.

Engineered nanosized cerium dioxide (CeO_2) materials are produced in approximately 10,000 tonnes per year and used in products as catalysts, fuel additives and cells, polishing agents, and semiconductors. The high and increased production combined with sparse availability of toxicological information and conflicting results have increased concerns for health effects in the human population. To improve understanding of CeO_2 nanoparticle toxic effects, information about their kinetics is vital. Such associations can be gained by use of physiologically based pharmacokinetic (PBPK) models. The aim was to develop a PBPK model to describe the biokinetics of cerium dioxide nanoparticles infused intravenously into rats. The model is based on a published nano PBPK model by Carlander and coworkers (International Journal of Nanomedicine 2016,11,625-40) and reflects findings in biodistribution studies on CeO_2 nanoparticles where the majority resides in organs belonging to the mononuclear phagocyte system. The model was calibrated and validated with data on 5 nm CeO_2 nanoparticles and shows good predictability. The most sensitive nanoparticle dependent model parameter is uptake to phagocytic cells. The applicability of the model for other sizes of CeO_2 nanoparticles remains to be tested.
Fundign provided by the Swedish Research Council for Health, Working Life and Welfare (Forte, grant No 2010-0702), Sweden and the NANoREG project of the European Union Seventh Framework Programme (grant No 310584).

Keywords: Biodistribution, Biokinetics/toxicokinetics, Cerium oxide nanoparticles

11. In vivo genotoxic effects of uncoated and coated CeO2 NPs administrated to mice by pharyngeal aspiration

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Ceria nanoparticles (CeO_2 NPs) have several industrial applications and pharmacological potential due to their antioxidant properties. However, toxicity data on CeO_2 NPs are scarce and show contradictory results. In the present study, we examined whether uncoated, polyethylene glycol- and citrate-coated CeO_2 NPs (4-8 nm; PlasmaChem), administrated by repeated dose (3x) pharyngeal aspiration, could be genotoxic to mice, locally in the lungs or systemically in the liver, peripheral blood and bone marrow. C57Bl/6 mice were treated with four different doses of each NPs (corresponding to 4.4, 8.8, 17.6 and 35.2 µg Ce2+/mouse/aspiration), and sampled 1 and 28 days after the last administration. DNA damage was assessed by the comet assay in bronchoalveolar lavage (BAL), lung and liver cells. Micronuclei, a biomarker of chromosome damage, were analysed in bone marrow and peripheral blood erythrocytes. Furthermore, histopathological effects on the lungs and biodistribution of the NPs (analysis of Ce2+ in several organs) were assessed. At 24-h, a significant increase in DNA damage was induced by all the NPs in BAL cells but not in lung cells. No systemic genotoxic effects in the liver or bone marrow were observed. A dose-dependent accumulation of macrophages and activated lymphocytes was seen in the lungs for all the NPs, although a milder reaction was elicited by the coated NPs. Our findings show that short-term exposure of mice to CeO_2 NPs induces DNA damage in BAL cells, but not in lung cells or systemically, and pulmonary inflammation (Funded by the EU FP-7 GUIDEnano, Grant Agreement No. 604387).

Keywords: Cerium oxide nanoparticles, Genotoxicity, In vivo toxicology, Metal/metal oxide nanomaterials

12. Role of Physicochemical Properties of Gold Nanoparticles on Biocorona Formation and Cellular Uptake Profiles in Endothelial Cells

Parwathy Chandran, Nancy A. Monteiro-Riviere. Nanotechnology Innovation Center of Kansas State, Kansas State University, USA.

The formation of a protein corona (PC) over nanoparticles (NP) upon entry into the systemic circulation can alter NP-cell interactions, uptake, and biodistribution; subsequently determining their fate in vivo. It is unclear about the role of NP physicochemical properties in governing the PC, and its effect on cell uptake. This study investigated the role of size and surface chemistry on PC formation and effect on uptake using 40nm and 80nm gold NP (AuNP) with branched polyethyleneimine (BPEI), lipoic acid (LA) and polyethylene glycol (PEG) coatings in human umbilical vein endothelial cells (HUVEC). Gel electrophoresis of hard corona proteins of individual AuNP incubated in plasma for 1h showed surface functionalization dependent signature adsorbomes exhibiting human serum albumin (HSA) enrichment. Time-dependent uptake of bare, plasma corona and HSA corona bound AuNP at 0.25, 0.5, 1, 3, 6, 12 and 24h using ICP-MS showed size and surface chemistry dependent uptake patterns. Bare 40nm AuNP showed greater uptake compared to 80nm AuNP, and cationic BPEI-AuNP showed highest uptake, followed by anionic LA and PEG-AuNP. The presence of a complex plasma corona reduced internalization drastically, where the effect was most pronounced with BPEI and LA-AuNP. A simple HSA corona increased the uptake of BPEI, LA and PEG-AuNP significantly, compared to plasma corona-AuNP. Hyperspectral imaging and TEM confirmed the patterns. Establishment of definite links between NP physicochemical properties, protein corona profiles, and cell uptake is beneficial for the rational design of NP for biomedical applications. Funding provided by the Nanotechnology Innovation Center of Kansas State.

Keywords: Biocorona, Physicochemical characterization, Gold nanoparticles, Endocytic uptake

13. Absolute Identification and Quantification of DNA Repair Proteins: An Emerging Measurement Trend in Nanotoxicology

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Oxidatively induced DNA damage is repaired in vivo by various mechanisms involving numerous DNA repair proteins. Persistent DNA damage may lead to mutagenesis, which is mechanistically fundamental to all cancers. Due to unique surface-active properties of engineered nanomaterials (ENM), it is important to understand and characterize their molecular-scale interactions with biomolecules inside cells. Characterizing and quantifying the effects of ENMs on endogenous DNA repair protein expression levels is an emerging area of nanosafety research. We developed a methodology to identify and accurately measure DNA repair proteins in human cells and tissues using LC-MS/MS. We measured hAPE1 and hMTH1 levels in human normal and malignant breast tissues, and in human cultured cell lines, some of which were exposed to superparamagnetic iron oxide nanoparticles. We also developed the methodology to identify and measure other DNA repair proteins such as NEIL1, NTH1, OGG1, PARP1 and Pol β. The use of ^{15}N-labeled analogs of these proteins as internal standards is critical for their accurate measurement, so we generated ^{15}N-labeled versions of these proteins to be used as internal standards. These standards were added to nuclear or cytoplasmic protein extracts isolated from cells, followed by hydrolysis of the extracts with trypsin, and LC-MS/MS analysis to achieve positive identification and absolute quantification of protein levels. This novel methodology may help improve our understanding of protein-nanoparticle interactions. More specifically, it may serve as a reference procedure for the accurate measurement of endogenous DNA repair protein expression levels following exposure to ENMs or other agents of interest.

Keywords: Genotoxicity, In vitro toxicology

14. Short-term Inhalation Exposure to Copper Oxide Nanoparticles Induces Gene Expression Changes Associated with Inflammation and Cell Proliferation in the Rat Bronchoalveolar Epithelium

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Copper oxide nanoparticles (CuO NPs) are considered for many applications, from electronics to antimicrobial agents, leading to growing concerns about potential human health hazards. Here, inhalation exposure of rats for five consecutive days to two doses of CuO NPs, 3.3 (low dose, LD) and 13.2 (high dose, HD) mg/m^3 was performed and lungs were collected on days 6 and 28. Histopathology revealed inflammation that was resolved during the post-exposure period. Global microarray analyses yielded about 1,000 differentially-expressed probes in HD rats and 200 in LD, contrasting with <20 after recovery. Pathway analysis indicated cell proliferation/survival and inflammation as the main processes triggered by exposure and identified epithelial cell transforming protein 2 (Ect2) as a potential gene of interest implicated in cell proliferation. Indeed, Ect2 was upregulated in exposed lungs and was immunolocalized in the cytoplasm of broncho-epithelial cells in hyperplasic foci in HD rats. Monocyte chemoattractant protein 1 (MCP-1/CCL2) was also upregulated and this was corroborated immunohistochemically. The findings suggest that airborne CuO NPs cause an acute response that translates into inflammation and cellular proliferation in bronchoalveolar epithelium, with upregulation of neoplasia-related factors even after a short-term exposure. While lung inflammation is in accordance with our recent studies, microarray analysis identified novel lung responses to CuO NPs that would otherwise have been overlooked.

Keywords: In vivo toxicology, Metal/metal oxide nanomaterials, Systems biology/toxicology

15. Cerium Oxide Nanoparticles Preserve Muscle Function and Increase Longevity in the SOD1G93A Mouse Model of Amyotrophic Lateral Sclerosis

William DeCoteau, Karin Heckman, Ana Estevez, Paige Studlack, Jennifer Clauss, Elizabeth Nichols, Jennifer Lipps, Matthew Parker, Bonnie Hays-Erlichman, James Leiter, Joseph Erlichman. St. Lawrence University, USA.

Oxidative stress is a key contributor to the motor neuron loss and clinical progression of Amyotrophic Lateral Sclerosis (ALS), and in the SOD1G93A transgenic mouse model of ALS. Cerium oxide nanoparticles (CeNPs) are potent superoxide dismutase mimetics. Here we tested the hypothesis that treatment with custom-made CeNPs (20 mg/kg) injected twice a week at the onset of motor weakness would protect SOD1G93A mice from further muscle weakness and, ultimately, prolong their survival. Motor function was monitored by measuring the latency to fall from a hanging wire grip strength test. Results indicated that the rate of decline in the CeNP treated animals was significantly slower in both males and females compared to control mice. We also found the overall decline averaged across the first 25 days of treatment to be significantly worse in control animals compared to the male and female mice treated with CeNPs. Furthermore, we found the median estimated survival after the onset of treatment was significantly longer in mice given CeNPs (33 days) compared to mice treated with vehicle (22 days). Thus, SOD1G93A mice receiving CeNPs from the onset of motor signs survived significantly longer than the control animals. Together, these results suggest CeNPs hold promise as an anti-oxidant therapy for the treatment of ALS and other neurodegenerative disorders.

Keywords: Biomedical/therapeutic applications, Cerium oxide nanoparticles

16. Advanced Computational Modeling for In Vitro Nanomaterial Dosimetry

Glen DeLoid[1], Joel Cohen[1], Georgios Pyrgiotakis[1], Sandra Pirela[1], Anoop Pal[1], Jiying Liu[2], Jelena Srebric[3], Philip Demokritou[1]. [1]Harvard T.H. Chan School of Public Health; [3]University of Maryland, USA; [2]Shandong Jianzhu University, P.R. China.

Accurate dose metrics are a basic requirement for in vitro safety assessment of engineered nanomaterials (ENMs). Recently, hybrid experimental and modeling platforms have been developed to quantify what cells "see," during an in vitro exposure. Such approaches require standardized ENM suspension preparation, accurate characterizatoin of agglomerate sizes and effective densities, and predictive fate and transport modeling. Here we present results from, and validation of, two robust computational transport models. Both three-dimensional computational fluid dynamics (CFD) and a newly-developed Distorted Grid (DG) model were used to predict concentration and deposition metrics for industry-relevant metal oxide ENMs in cell culture media. Both models accomodate the complete size distribution of agglomerates. The DG model also allows for ENM dissolution over time, and models adsorption of ENMs at the cellular interface as a Langmuir isotherm goverened by a dissociation constant, KD. The two models were validated and found in remarkably close agreement using quantitative analysis of flash-frozen, cryosectioned columns of ENM suspensions. Results of simulations based on agglomerate size distributions differed substantially from those obtained using mean sizes. The effect of adsorption were negligible for KD values greater than 1 nM, whereas smaller values resulted in more rapid and complete deposition of material, suggesting that a reflective lower boundary condition is closer to reality than a sticky boundary. These advanced models in conjuction with proper suspension preparation and characterization can provide practical and robust tools for obtaining accurate dose metrics for high-throughput screening of ENMs. Funding provided by NIEHS grant (ES-0000002), NSF grant (ID 1436450).

Keywords: Dosimetry, Hazard ranking/characterization, In silico modeling, In vitro toxicology, Metal/metal oxide nanomaterials, Physicochemical characterization, Risk assessment

17. Emerging Tools and Approaches for Bridging the Gap between Exposure and In-vitro/In-vivo Dosimetry of Engineered Nanomaterials for Nanosafety Assessment

Philip Demokritou[1], Joel Cohen[2], Gled DeLoid[1]. [1]TH Chan School of Public Health, Harvard University; [2]Gradient Corp., USA.

Because of the potential public health risk posed by the rapidly expanding array of engineered nanomaterials (ENMs) currently in use in many consumer products and industrial applications, high-throughput in vitro methods for safety assessment are sorely needed, but to date have not proven reliable in predicting toxicological outcomes in animal models. One of the important reasons for this discordance is the failure to reconcile in vitro with in vivo doses and link them to "real world" ENM exposure levels. Furthermore, despite growing evidence of the importance of dosimetry in in vitro hazard ranking, very few studies take it into consideration. This may be primarily due to a lack of standardized, easy to use, and validated tools and methodologies for dispersion preparation, characterization and in vitro dosimetry estimation. This talk will present the importance of measuring cellular dosimetry of ENMs and its implications for the field of nanomaterial safety, and highlight emerging integrated experimental/numerical dosimetric methodologies that can be used in nanotoxicology field with specific examples related to "real world" ENM exposures.

Keywords: Dosimetry, In vitro toxicology, In vivo toxicology

18. Adaptation of the OECD Guidelines to Support Nanogenotoxicity Hazard Assessment

Shareen Doak, Swansea University, UK.

With the growth of the nanotechnology industry and the wide array of nano-containing consumer products being developed, there is increasing pressure to define a hazard identification and risk management strategy for nanomaterials to prevent stifling innovation. Consequently, a battery of appropriate in vitro assays assessing several genotoxicity endpoints is required to minimise extensive and costly in vivo testing. However, the validity of using the established protocols in current OECD recognised genotoxicity assays is under scrutiny for nanomaterials because their unique physico-chemical properties can result in unexpected interactions with experimental components that generate misleading data-sets. Caveats to investigating the genotoxicity of nanomaterials have now become evident and demonstrate the need for a systematic approach to develop a robust in vitro genotoxicity testing strategy. This is essential to ensure consistency and reliability when evaluating nanomaterials. Thus, specific recommendations to facilitate such standardisation coupled to novel approaches to enhance in vitro test systems need to be more widely implemented.

Keywords: Genotoxicity

19. Advances In Our Understanding of Nanomaterial Carcinogenicity

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There are considerable gaps in our knowledge of the carcinogenic potential of nanomaterials (NMs). The testing strategy for genotoxicity of NMs covers important endpoints such as DNA damage, mutations, chromosomal aberrations and clastogenicity. While genotoxic carcinogens are readily recognised, the situation is more difficult for non-genotoxic carcinogens. Although many studies have investigated genotoxicity of NMs in vitro, there are few studies of carcinogenicity using in vitro approaches. The classification of non-genotoxic NMs as carcinogenic in vitro (without confirmation in animal studies) is only credible if the in vitro assays have been validated and adequate knowledge about the mechanisms is available. The most promising model systems for detecting morphological neoplastic transformation potential of NMs are based on Syrian Hamster Embryo (SHE), C3H10T1/2 and Bhas42 cell transformation assays (CTA). We used Bhas42 cells to study the transforming ability of several types of NMs including carbon nanotubes, titanium dioxides, silver nanoparticles and iron oxides. Results show different effects of NMs of the same chemical composition, suggesting that other physicochemical properties might play a role in the differences between the NMs and in the mechanisms of their toxicity. Nano-specific properties such as size, surface reactivity, crystallinity, shape, solubility, aggregation and agglomeration including changes of the NM behavior in environmental media must therefore be considered. The use of alternative toxicological tools such as CTA, genome-wide expression profiling and in vitro high-throughput and high content analysis is important for understanding the underlying mode-of-action of NMs. Supported by FP7NANoREG (NMP4-LA-2013-310584), and Norwegian Research Council project NorNANoREG (239199/O70).

Keywords: Carcinogenicity, Genotoxicity, In vivo toxicology, Risk assessment

20. Nanomaterial genotoxicity testing

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Nanomaterials (NMs) have unique properties owing to their small size and relatively large surface area. As more and more NMs are introduced into FDA-regulated products, there is rising concern for their safety assessment. Methods for NM assessment are complicated by sample preparation and biological test environments. There is growing consensus in the genetic toxicology community that methods for the standard genotoxicity assays are not adequate for assessment of NMs. Problems include: the general lack of uptake of nanoparticles into bacteria; the inhibitory effect of cytochalasin B (a standard component of the in vitro micronucleus assay) on uptake of particles into mammalian cells; the interference of NM particulates with cell suspension assays such as the mouse lymphoma assay; physical interference of some NM particles with the mitotic spindle; and the generally weak effects observed leading to lack of consensus on test results. The genetic toxicology community, including scientists from academia, regulatory agencies and industry, is working to develop recommendations for genotoxicity testing of NMs, including modifications of the existing test methods and a revised test battery that alters the usual selection of in vitro and in vivo assays. The diversity of NMs and the likelihood of secondary effects, rather than direct damage to DNA, result in considerable challenges in the assessment of human risk.

Keywords: Regulatory decision making, regulatory decision making, genotoxicity, alternative testing methods/strategies

21. Targeting Nanoparticles to Brain: Impact of N-methyl D-aspartate Receptors

Ayse Basak Engin. Gazi University, Turkey.

Neurodegenerative disorders are amongst the major debilitating conditions of our century. Activation of brain microglia may promote neuronal injury and death through production of glutamate, pro-inflammatory factors, reactive oxygen species, quinolinic acid. It has recently been shown that nanotechnology greatly facilitated diagnosis and treatment of central nervous system diseases. Although the causes of dopaminergic neuronal damage remain unknown, to slow down or halt this process, nanotechnology based therapeutic perspectives are open to debate. In this presentation, the impact of more specific drug targets which interact with dopaminergic neuron receptors will be discussed. As liposomes, gelatin-cored nanostructured lipid carriers have high stability, strong penetration, encapsulating efficiency, loading capacity as well as bioactivity, they are excellent for targeted delivery. Attachment of superoxide dismutase and anti-glutamate N-methyl D-aspartate (NMDA) receptor 1 antibody to poly(butyl cyanoacrylate) nanoparticles increased their neuroprotective efficacy. Nanotization of nicotine, as well as nicotine-encapsulated poly(lactic-co-glycolic acid) (PLGA) nanoparticles prevented the dopaminergic neurodegeneration and apoptosis that is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors. Also, caffeine-encapsulated PLGA nanoparticles exhibited higher protective effect on dopaminergic neurons. Additionally, administration of dopamine by loaded PLGA nanoparticles reversed neurochemical and neurobehavioral deficits in neurodegenerative diseases. Furthermore, NMDA receptors were more sensitive to the red wine polyphenol. In this context, resveratrol-loaded polysorbate 80-coated poly(lactide) nanoparticles had neuroprotective effects on dopaminergic neurons. Regarding these findings, in our studies, we observed that NMDA receptors over-activation is linked to neurodegeneration. Thus, targeting nanostructured materials to NMDA receptors for biomedical and pharmaceutical applications may be considered as treatment options.

Keywords: Biomedical/therapeutic applications, neurodegeneration

22. Measurement of catalase and oxidase activity of custom-synthesized cerium oxide nanoparticles and assessment of their antioxidant effectiveness in a mouse hippocampal brain slice model of ischemia

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Both therapeutic and toxic effects of cerium oxide nanoparticles (CeNPs) have been reported. An understanding of the chemical and biological factors that underlie these disparate findings is vital if the ultimate goal is to use CeNPs clinically. Here, we characterize the effects of custom-synthesized CeNPs stabilized with EDTA and citrate. These CeNPs had a negative zeta potential (-22.94 ± 1.07 mV), showed polydispersity, were small in diameter (4.4 nm), and displayed a diffraction pattern consistent with a fluorite lattice structure. We assayed catalase and oxidase activity in a cell-free system utilizing a commercially available kit and observed high levels of catalase activity, comparable to those of the benchmark antioxidant N-acetylcysteine (NAC). The CeNPs also displayed significantly lower levels of oxidase activity compared to NAC. CeNPs (5.8 µM) significantly reduced tissue death in a mouse brain slice model of ischemia. The levels of CeNP-mediated neuroprotection were comparable to protection conferred by a dose of NAC (10 mM) that was ~1700 time higher. Finally, we measured the effects of CeNPs on static oxidation-reduction potential (sORP) and resistance to oxidative stress in brain tissue. Compared to vehicle-treated controls, CeNPs prevented the oxidizing shift associated with exposure to simulated ischemia or H_2O_2. Taken together, these data demonstrate that our custom-synthesized CeNPs displayed potent antioxidant activity in biological settings.

Keywords: Cerium oxide nanoparticles

23. Contrasting Hazard of Emerging Nanomaterials Across a Range of Species and Endpoints – Lessons for Nanosafety

Teresa F. Fernandes, Heriot-Watt University, UK.

The interest in the effects of engineered nanomaterials (ENMs) in the scientific literature is continuing to grow along with the increasing number of applications of nanotechnology. Although availability of data continues to increase the focus still on a narrow range of species with little attempts to compare effects across species and how exposures in realistic environmental conditions may impact on bioavailability and hazard. In this paper the results of exposures of a range of nanomaterials, with different coatings, will be compared across different environmental conditions and species. This presentation will provide a summary of the state of the art on the occurrence of specific nanomaterial effects in biological systems; differentiate cells/tissue injuries due to nano and non-nano materials and between nanomaterials with similar chemistry but different physical properties, comparing studies of pristine nanomaterials in static conditions with more realistic scenarios. Results from this work contribute to current developments in the field including the derivation of read-across and cross taxa and endpoints comparisons.

Keywords: Environmental nanotoxicology, cross species effects, extrapolation, realistic scenarios, coatings

24. A Combined Proteomics and Metabolomics Approach to Assess the Effects of Gold Nanoparticles In Vitro

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Omics technologies, such as proteomics or metabolomics, have to date been applied in the field of nanomaterial safety assessment to a limited extent. To address this dearth, we developed an integrated approach combining the two techniques to study the effects of two sizes, 5 and 30 nm, of gold nanoparticles (AuNPs) in Caco-2 cells. We observed differences in cells exposed for 72 h to each size of AuNPs: 61 responsive (up/down-regulated) proteins were identified and 35 metabolites in the cell extract tentatively annotated. Several altered biological pathways were highlighted by integrating the obtained multi-omics data with bioinformatic tools. This provided a unique set of molecular information on the effects of nanomaterials at cellular level. This information was supported by complementary data obtained by immunochemistry, microscopic analysis and multiplexed assays. A part from increasing our knowledge on how the cellular processes and pathways are affected by nanomaterials (NMs), these findings could be used to identify specific biomarkers of toxicity or to support the safe-by-design concept in the development of new nanomedicines. This work was supported by the European Commission Joint Research Center (JRC).

Keywords: Alternative testing methods/strategies, Human toxicology, In vitro toxicology, Systems biology/toxicology, Toxicological mechanisms, Omics, Nanotoxicology

25. Fate of Cerium Ions in the Lungs: In vivo Formation of Cerium Phosphate Nanoparticles from Instilled Cerium Chloride

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The higher lung retention of Ce after instillation of $CeCl_3$ (75-92% at 28 days) is unexpected since metal ions are readily transported across the air-blood barrier. Poorly described mechanisms may be involved such as Ce ion binding to lung constituents or formation of precipitates. Determining the form of Ce after lung exposure to $CeCl_3$ has been challenging due to the difficulty in distinguishing ions from particulate forms. Here we demonstrate the in vivo formation of nanoparticulate Ce-containing structures in lungs instilled with 5 mg/kg $CeCl_3$ using TEM with electron energy loss spectroscopy (EELS). The lungs were fixed by vascular perfusion with 2.5% glutaraldehyde, 2% paraformaldehyde in 0.1M HEPES buffer, and processed for electron microscopy at 2 hours and 7 days post-instillation. High resolution 2D/3D imaging and EELS revealed in vivo formed crystalline Ce-phosphate nanoparticles at both time-points after $CeCl_3$ exposure in the lysosomal regions, along membrane surfaces and inside mitochondria. We observed co-localization of Ce and P with EELS spot analyses taken on individual nanoparticles. EELS regional maps demonstrated P-rich zones in the vicinity here nanoparticle formation occurred. These Ce-phosphate nanoparticles were on the order of 1-4 nm and aligned into rod-shaped structures of ~10 nm long and 1-4 nm wide. Moreover, the Ce-phosphate rods formed ~50-300 nm clusters. In vivo formation of Ce-phosphate nanoparticles from Ce ions were comparable to those observed during bioprocessing of cerium oxide. This suggests similar in vivo particle retention mechanisms and may explain the long half-life of Ce ions in the lungs.

Keywords: In vivo toxicology, bioprocessing, ion transport

26. Contrasting the in vivo Processing Variances of Nanoceria in Organs: Major Differences between Spleen and Liver Revealed

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Site-specific accumulation, retention, bioprocessing and clearance of nanoparticles (NPs) by various organs remain formidable challenges to understand the intricate drivers that control nanotoxicity. Determining the in-vivo processing of NPs at the cellular and subcellular levels will remain a distant reality unless we can unravel the complex 2D/3D architecture, cell specific endpoints, catalytic reactivity and electronic state of NPs inside target organs, while focusing on the NP's complex physical, chemical and biological interactions. Extensive deposition of nanoceria is greatest in the spleen followed by the liver after IV-administration of a single dose (85 mg Ce kg−1) of citrate-coated, aqueously dispersed NPs into rats. Our team has previously established the in-vivo processing pathways of these NPs inside the liver which results in 2nd generation of ultra-small (<4 nm) nanoceria and involves a dissolution-reprecipitation mechanism. After 90 days in the lysosomal regions, the transformed nanoceria are well dispersed and exhibit an increased number of defects which greatly promotes their free radical scavenging potential. We correlated the processing of nanoceria in the liver to a reversal in oxidative stress observed in the brain, indicating a transition from initially pro-oxidant to anti-oxidant effects. We will present data showing various degrees of subcellular internalization of nanoceria and reaction products in the spleen to contrast the distribution, size, composition, morphology and redox properties with those in the liver. The *in situ* analysis demonstrates how different environments in the spleen compared to the liver contribute to diverse NP degradation and bioprocessing phenomena. Support: EPA RD-833772 & R01GM109195.

Keywords: Biodistribution, Cerium oxide nanoparticles, Exposure characterization, In vivo toxicology, bioprocessing

27. Influence of Agglomeration and Specific Lung Lining Lipid/Protein Interaction on Short-term Inhalation Toxicity

Andrea Haase[1], Wendel Wohlleben[2], Marc D. Driessen[1], Simon Raesch[3], Ulrich F. Schaefer[3], Christine Schulze[3], Bernhard von Vacano[2], Antje Vennemann[4], Martin Wiemann[4], Christian A. Ruge[3], Herbert Platsch[5], Sarah Mues[6], Rainer Ossig[6], Janina Tomm[7], Jürgen Schnekenburger[6], Thomas A. J. Kuhlbusch[8], Andreas Luch[1], Claus-Michael Lehr[9]. [1]German Federal Institute for Risk Assessment (BfR), Department of Chemicals and Product Safety; [2]BASF SE Material Physics and [5]Biophysics & Proteomics; [3]Biopharmaceutics and Pharmaceutical Technology, Department of Pharmacy, Saarland University, [4]IBE R& D gGmbH, Institute for Lung Health; [6]Biomedical Technology Center, Westfälische Wilhelms-University; [7]Helmholtz Centre for Environmental Research (UFZ), Department of Proteomics; [8]Institute of Energy and Environmental Technology (IUTA) e.V., Air Quality & Sustainable Nanotechnology; [9]Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Germany.

Lung lining fluid is the first biological barrier nanoparticles (NPs) encounter during inhalation. Previous inhalation studies revealed considerable differences between surface functionalized NPs. Our aim was to investigate the influence of lipid and/or protein binding on that. We analyzed a set of surface functionalized NPs including different SiO_2 and ZrO_2 in pure phospholipids, CuroSurfTM and purified native porcine pulmonary surfactant (nS). Lipid binding was surprisingly low for pure phospholipids. Only few NPs attracted a minimal lipid corona. The hydrophilic surfactant protein A in nS facilitated lipid binding to all NPs. The degree of lipid and protein affinities for different surface functionalized SiO_2 NPs in nS followed the same order (SiO_2 Phosphate ~ unmodified SiO_2 < SiO_2 PEG < SiO_2 Amino NPs). Agglomeration and biomolecule interaction of NPs in nS was mainly influenced by surface charge and hydrophobicity. Differences in short-term inhalation studies were mainly influenced by the core composition and/or surface reactivity of NPs. However, agglomeration in lipid media and lipid/ protein affinity appeared to play a modulatory role on inhalation toxicity. Thus, our results may have an influence on planning meaningful in vitro toxicity tests.

Keywords: Alternative testing methods/strategies, Biocorona

28. Omics, Bioinformatics and Adverse Outcome Pathways in Nano Safety Research

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Transcriptomics, the systematic study of mRNA expression employs global expression profiling tools, such as microarrays to identify and quantify transcripts that are differentially expressed following exposure of an organism or isolated cells to chemicals. Transcriptomics is an important systems biology tool that has been widely used to understand the underlying mechanisms of chemical/nanomaterial induced toxicity, categorise chemicals, disease states, tissue or strain specific responses and transcriptomics data is being evaluated for its applicability to inform human health risk assessment of toxic substances. However, the challenge lies in the effective use of these data to deriving biologically meaningful networks of genes that are reflective of disease phenotypes or adverse outcomes of regulatory importance. This presentation will demonstrate how transcriptomics data was used to establish a mechanisms-based adverse outcome pathway (AOP) for multi-walled carbon nanotubes (MWCNT)-induced lung fibrosis, discern various key events that are essential in the process leading to lung fibrosis, inform data gaps for development of alternative (in vitro, ex vivo) assays that can be used to screen novel nanomaterials for their potential to induce lung inflammation and fibrosis, and derive transcriptional/pathway benchmark dose values that can be applied to read across and quantitative risk assessment of nanomaterials.

Keywords: Adverse outcomes pathway analysis, Carbon-based nanomaterials, Systems biology/toxicology

29. Transformations and Biological Impact of Emerging Energy Storage Nanomaterials

Robert Hamers, University of Wisconsin-Madison, USA.

The rapid increase in mobile electrons and electronic vehicle technologies is leading to a rapid escalation in the use of complex oxides as cathode materials in the lithium-ion batteries that power these devices. Economic factors are driving a trend toward mixed-oxide materials such as LixNiyMnzCo1-y-z O_2 ("NMC") that combine high performance with low cost. However, these materials also incorporate substantial amounts of metals such as Ni and Co that may post environmental risk, and there is not current any national infrastructure for recycling of these materials. We have been investigating the transformation of these emerging nanomaterials and the resulting biological impact as revealed through acute and chronic mortality studies and gene expression studies using Shewanella oneidensis and Daphnia magna as model organisms. Further molecular-level insights are provided by detailed investigations of NMC interactions with supported lipid bilayers. Our results show that this class of materials undergoes incongruent dissolution, preferentially releasing Ni^{2+} and Co^{2+} and leaving behind a Mn-rich transformation product. Exposure to NMC induces toxic effects through multiple pathways; with Shewanella these effects can be attributed almost exclusively to the redox dissolution of the NMC to form Ni^{2+} and Co^{2+} ions in solution. In contrast, ion-equivalent controls cannot reproduce the effects observed with Daphnia magna. These results highlight the need to develop a mechanistic understanding of the transformation of nanomaterials in the environment and the resulting impacts. Some perspectives on potential strategies for redesign to reduce adverse biological impact will be presented.

Keywords: Environmental nanotoxicology, Life cycle analysis, Metal/metal oxide nanomaterials

30. Acquired Superoxide-scavenging Ability of Ceria Nanoparticles

Xiao He, Yuanyuan Li, Zhiyong Zhang, Yuliang Zhao. Institute of High Energy Physics, P.R. China.

Ceria nanoparticles (nanoceria) are well known as a superoxide scavenger. However, inherent superoxide-scavenging ability was only found in the nanoceria with sizes 5 nm. We proved that the nanoceria with sizes >5 nm, different shapes, and negligible Ce^{3+}/Ce^{4+} ratio could acquire remarkable superoxide-scavenging ability via electron transfer. Our method will make it possible to develop nanoceria-based superoxide-scavenger with long-acting activity and tailorable nanoproperties.

Keywords: Cerium oxide nanoparticles, Cerium oxide nanoparticles, superoxide-scavenging, electron transfer

31. Scenario-Driven Tiered Nanotoxicology for Terrestrial Ecosystem Exposure and Hazard Assessments

Patricia Holden[1], Hilary A. Godwin[2], Jorge L. Gardea-Torresdey[3], Roger M. Nisbet[1], Yuan Ge[4], Monika Mortimer[1], Ying Wang[1], John H. Priester[5], Joshua P. Schimel[1]. [1]University of California, Santa Barbara; [2]University of California, Los Angeles; [3]University of Texas, El Paso; [5]Tulane University USA; [4]Chinese Academy of Sciences, P.R. China.

The University of California Center for Environmental Implications of Nanotechnology (UC CEIN), in conducting ecological research, develops and applies tiered approaches to assess exposure to and hazards of engineered nanomaterials (NMs) to terrestrial ecosystems. NM release scenarios and fate modeling establish realistic exposure regimes that influence conditions of tiered hazard assessment. Base food web receptors include heterotrophic and N2-fixing bacteria, and the primary producers, photosynthetic plants. Biological trophic transfer and the potential for biomagnification of bioaccumulated NMs initiate with protozoan and insect predators, respectively, and are studied as NM biological transport vehicles. In Tier 1, parallel bacterial and plant population growth studies define dose-response and bioaccumulation patterns for bioavailable NMs. Single-celled microbial studies are miniaturized for high throughput (HT) research, including of NM effects mechanisms for NM property-based variants. In Tier 2, microbial community composition and diversity shifts allow for assessing NMbioavailability in soil. In Tier 3, studies of the soil-grown plant system, including root symbioses, are conducted in pot-scale agricultural mesocosms, encompassing microbial-soil-plant-symbiosis interactions. Mathematical models of receptor growth and multi-receptor feedbacks, subject and responding to NM stresses, are populated by Tier 1 and 3 findings rega rding damage mechanisms and magnitudes. We illustrate the approaches, using results from researching hazards at cellular, population, community, and ecosystem scales for metal (Ag, and Cu), metal oxide (TiO_2, ZnO, CeO_2), quantum dot (CdSe), and carbonaceous (MWCNT, carbon black, graphene) NMs.

Keywords: Alternative testing methods/strategies, Carbon-based nanomaterials,Environmental nanotoxicology

Metal/metal oxide nanomaterials

32. Phagolysosomal membrane permeability and inflammasome activation as host susceptibility factors in ENM-induced lung inflammation

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There is increasing information on the mechanisms that may be involved with engineered nanoparticle (ENM)-induced lung inflammation including the important role of the NLRP3 inflammasome with the release of IL-1b and IL-18 in lung inflammation. However, the precise mechanisms of action leading to NLRP3 inflammasome activation have yet to be defined, although there is increasing evidence for the initial requirement of phagolysosomal membrane permeability as an upstream regulator of the NLRP3 inflammasome. In addition, mechanisms to account for genetic variance in inflammatory responses to ENM are also uncertain. In order to address these questions studies were conducted using ENM with isolated alveolar macrophages (AM) from C57Bl/6 and Balb/c mice and in vivo studies were conducted using the same strains. In vitro exposure demonstrated that Ag ENM caused greater toxicity and IL-1b release from Balb/c that C57B/6 AM. The difference could not be explained by uptake of the Ag ENM since the rate of uptake was higher in C57Bl/6 AM than Balb/c AM. In vivo studies demonstrated higher levels of a series of cytokines in lung lavage fluid (IL-1b, TNF-a, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70 and KC/GRO) in Balb/c compared to C57Bl/6 mice. Only INFg was higher in C57Bl/6 lavage fluid. Measurement of lysosomal membrane permeability using bone marrow-derived macrophages and AM from both strains demonstrated that Balb/c were more sensitive than C57Bl/6. There findings provide a potential explanation for the increased sensitivity of Balb/c mice compared to C57Bl/6 mice and may provide a focus for additional strain comparison studies.

Keywords: Metal/metal oxide nanomaterials, Susceptibility, inflammasome

33. Biological and Environmental Transformations of Emerging 2D Materials and Nanohybrids

Robert Hurt, Zhongying Wang, Annette von dem Bussche, Agnes Kane. Brown University, USA.

Nanotoxicology research has focused on a relatively small set of first-generation nanomaterials to elucidating the basic scientific principles governing biological responses. In parallel, the nanosynthesis field has moved forward quickly to develop functional hybrids, active structures, and next-generation nanomaterials. Among these are 2D materials, which are near-atomically-thin, high-aspect-ratio sheet-like solids with enormous variation in chemical composition and crystal phase. This talk focuses on chemical transformations of these emerging 2D materials and nanohybrids. We present a thermodynamic analysis, which together with iogeochemical data and new studies from our laboratory, show that a wide range of 2D nanosheets will undergo dissolution under conditions relevant to biological systems and the environment. Many 2D materials undergo dissolution through oxidation (e.g. transition metal dichalcogenides such as MoS_2, $MoSe_2$), while others undergo reductive dissolution (MnO_2 and MoO_3) in biological settings through the action of endogenous reducing agents. For these dissolving systems, the biological response is best understood in terms of the chemical toxicity of the soluble transformation products mediated by the timing and location of their release from the nanosheet phase. In practice, 2D materials are often combined with 0D particulate nanostructures to form functional hybrids, in which the nanosheets serve as catalyst supports, conductive additives, barriers, or scaffolds. The talk will describe a case study on nanohybrids fabricated from 2D graphene and 0D particles that illustrates complex dissolution and redox behavior arising in such multicomponent structures. Financial support from the National Institute of Environmental Health Sciences and the U.S. National Science Foundation are gratefully acknowledged.

Keywords: Composite nanomaterials, Emerging nanomaterials, composite nanomaterials, toxicological mechanisms

34. Differential Susceptibility in Humans and Rodent Models of Nanomaterial Toxicity or Lack Thereof

Salik Hussain, Stavros Garantziotis. NIEHS/NIH, USA.

Multipleepidemiological and experimental studies have shown that individuals with pre-existing inflammatory conditions are more prone to the adverse effects of environmental injury caused by particulate air pollution. However, only limited experimental data exist on susceptibility factors to engineered nanomaterial-induced toxicity. In order to address this knowledge gap, we performed ex-vivo exposures in primary human cells isolated from healthy individuals or subjects with pre-existing inflammatory disorders. We used nanoceria, nanotitanium dioxide and multi-walled carbon nanotubes as model exposures and evaluated toxic as well as inflammatory responses. Moreover, the role of single cell vs. co-culture and undifferentiated vs. differentiated cell exposure was determined. We demonstrate that pre-existing inflammatory conditions (such as asthma and metabolic syndrome) alter cellular susceptibility to engineered nanomaterials by affecting the magnitude or duration of response. Our data suggest a significant impact of exposure timing and sequence (in the case of co-exposure to nanomaterial and immune agonist) in cellular toxicity and inflammation. We also demonstrate that differentiated air-liquid cultures of human bronchial epithelia are resistant to nanomaterial-induced toxicity but mount robust inflammatory responses even at very low exposure levels. Our results validate the utility of more physiological relevant primary human cell modals and exposures to predict nanomaterial toxicity. Moreover, these findings exhibit the crucial need of more translational studies to evaluate the impacts of nanomaterials in individuals with pre-existing inflammatory disorders. Propose warrant the need Funding Statement: Intramural Research Program of the NIH, National Institute of Environmental Health Sciences (NIEHS).

Keywords: Alternative testing methods/strategies, Human toxicology, Susceptibility, Systems, biology/toxicology

35. Nanotechnology, a “Sustainable and Challenging” Innovation for Green Economy

Ivo Iavicoli[1], Veruscka Leso[2]. [1]University of Naples Federico II Department of Public Health Section of Occupational Medicine; [2]Catholic University of Sacred Heart Institute of Public Health, Italy.

The unique properties of nano-scale materials have made them attractive for a number of innovative, energy efficient, as well as economically and environmentally sustainable solutions to face a series of social challenges in a “green economy” perspective. However, the benefits of nano-enabled applications should be carefully balanced with the potential impact they may have on the society, particularly in terms of environmental, public and occupational health. Therefore aim of this presentation will be to assess potential fields of application for green nanotechnology with due regard to practical challenges, and potential risks for the general and occupational exposed populations. Smart energy nanotechnology can improve power delivery and storage systems, while reducing pressure on raw fossil materials and greenhouse gas emissions. Nano-based techniques in the agricultural field may implement delivery systems for agrochemicals and devices to detect and remove emerging pollutants and contaminants from water and soils. Additionally, strength, durability, heat-insulating, and self-cleaning properties of building materials may be improved by nanotechnology, while reducing their environmental impact. However, uncertainties concerning the release and fate of nanomaterials in the environment, their toxicological behavior and mechanisms of biological reactivity, and the difficulties in characterizing exposure levels should be overcome to reach a responsible green nanotechnology development. In this context, scientific, governmental and workforce efforts should be pursued for processes of opinion forming and decision making in green nanotechnology, in order to plan a precautionary and standardized risk management approach, especially in workplace settings, by identifying actual risks, preventive measures and suitable risk communication strategies.

Keywords: Occupational safety

36. How Does the Thickness of 2D Graphene Oxide Sheets Determine their Biological Fate and In Vivo Dosimetry?

Dhifaf Jasim[1], Herve Boutin[1], Michael Fairclough[1], Cécilia Ménard-Moyon[2], Alison Smigova[1], Christian Prenant[1], Alberto Bianco[2], Kostas Kostarelos[1]. [1]University of Manchester, UK; [2]University of Strasbourg, France.

The biodistribution of graphene oxide (GO) materials after intravenous (IV) administration in mice has been shown previously with major accumulation in the reticuloendothlial system, such as spleen, liver and lungs. Significant levels of urinary excretion have also been reported [1]. In this study, two graphene oxide materials were functionalized with a radiometal chelating moiety (DOTA) and labelled with 64Cu for positron emission tomography (PET) imaging. The two resulting graphene oxide materials had two distinct thickness distributions, f-GO-thin (5nm) and f-GO-thick (20nm). Our results revealed that 24h after intravenous administration in mice, the thicker GO sheets accumulated to a greater extent in the liver and spleen (47.5%) compared to the thinner ones (23.1%). The thin GO material exhibited excretion in the urine through the glomerular route up to 70% of the injected dose. This study provides important in vivo structure–activity relationships and an important understanding of the blood kinetic profile of 2 dimensional materials with different thickness distributions. Such work is thought to direct potential biomedical applications of such novel materials and determine their in vivo dosimetry levels in critical organs. Funding provided by the EU 7th RTD Framework Programme, Graphene Flagship project (FP7-ICT-2013-FET-F-604391) and the Wolfson Molecular Imaging Centre (University of Manchester). This work was also partly supported by the Centre National de la Recherche Scientique (CNRS) by the Agence Nationale de la Recherche (ANR).

References: [1] Jasim D. A., et al. Chemical Science (2015) 6, 3952-64.

Keywords: Carbon-based nanomaterials, Emerging nanomaterials

37. Has Nanotechnology the Potentialt to Make Modern Agriculture a Little Greener?

Melanie Kah. University of Vienna, Austria.

Researchinto nanotechnology applications for use in agriculture has become increasingly popular over the past decade, with a particular interest in developing so-called “nanopesticides”. Due to their direct route of release, nanopesticides may be regarded as particularly critical in terms of possible environmental impact, as they (would) represent the only intentional diffuse source of engineered nanoparticles in the environment. In addition, all pesticides are inherently toxic (at least to the target pest) and, thus, associated with some risk. Nanotechnology has however a lot to offer to the agrochemical sector with regard to the possible reduction of the impact on human and environmental health. The last couple of years have seen increasing incentives to use nanotechnology to develop products that may be less harmful to the environment relative to conventional agrochemicals. Possible benefits include increased efficacy, reductions in application rates, exposure to non-target organisms or the development of resistances. It is thus crucial that nanopesticides are assessed by looking at both the risks and benefits associated with their use relative to current solutions. After summarizing the current state of knowledge related to the development of nanopesticides, we will evaluate our current ability to provide such an assessment, in particular with regards to environmental exposure. Future directions will be suggested that may allow taking advantage of what nanotechnology could offer to support the development of greener plant protection products.

Keywords: Emerging nanomaterials, Exposure assessment, Regulatory decision making

38. Alternative Toxicity Testing of Nanomaterials Using 3D Human Lung Microtissues

Agnes B. Kane, Pranita K. Kabadi, April Rodd, Alysha Simmons, Robert H. Hurt. Brown University, USA.

The current standard for pulmonary toxicity testing of respirable, poorly-soluble engineered nanoparticles including carbon nanotubes is a 90-day inhalation assay in rodents. Alternative approaches using computational modeling and in vitro high content screening assays have been proposed as initial steps in a tiered testing strategy for engineered nanoparticles (Godwin et al., ACS Nano, 2015). We have developed a novel human 3D lung microtissue platform containing fibroblasts, epithelial cells, and monocyte-derived macrophages to investigate whether carbon nanotubes activate profibrotic pathways in vitro. Lung target cells self-assemble into 3D spheroids in scaffold-free agarose gels and remain viable for up to 21 days. After 4–7 days, sublethal doses of multiwalled carbon nanotubes induce fibroblastic foci similar to pulmonary histopathological changes that develop in mice (Katwa et al., Small, 2012). Increased gene expression for extracellular matrix proteins, matrix metalloproteinases, profibrotic mediators, and cytokines is found following exposure to carbon nanotubes in contrast to a similar dose of carbon black nanoparticles. This 3D human lung microtissue platform provides an alternative to traditional in vivo models in pulmonary nanotoxicology. This research is supported by an NIEHS Training Grant (T32 ES07272), a Superfund Research Program Grant (P42 ES013660), and a generous gift to Brown University by Donna McGraw Weiss ’89 and Jason Weiss.

Keywords: Alternative testing methods/strategies, Carbon-based nano materials

39. Genetic Determinants of Nanoparticle-Induced Lung Inflammation

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Engineered nanoparticles (ENPs) are important components of a variety of consumer products and the risks to humans involved in handling them during manufacture or use is difficult to assess. Importantly, in vivo studies conducted in rodents indicate that many ENPs can cause lung inflammation and toxicity in a strain- and particle type-dependent manner. Because many ENPs are known to elicit oxidative stress in biological systems, we have used mice with compromised glutathione (GSH) synthesis to model genetic polymorphisms in GSH synthesis in ENP disposition and toxicity. We have also used multiple inbred mouse strains to study the overall influence of genetics on ENP-induced lung inflammation and toxicity. The inflammatory potentials of multiwall carbon nanotubes (MWCNTs), quantum dots (QDs), and silver nanoparticles (AgNPs) are all dependent on genetic susceptibility factors. Surprisingly, C57BL/6 mice appear to be resistant to a number of ENPs. Multiple inbred strains of mice and genome-wide association (GWA) mapping identified potential candidate susceptibility loci for AgNP-induced lung inflammation on chromosomes 1, 4, 5 and 18. Quantitative real-time PCR qRT-PCR revealed significant inverse associations between mRNA levels for several genes in these loci and neutrophil influx into the lung. These data underscore the need to evaluate the toxic and inflammatory effects of ENPs in multiple mouse and rat strains. Doing so will improve ENP risk assessments that rely on intra- and interspecies adjustment factors. Supported by NIEHS Grants R01ES016189, U19ES019545, P30ES07033 and U01ES020126.

Keywords: Carbon-based nanomaterials, Metal/metal oxide nanomaterials, Susceptibility

40. Silica Coating Influences the Corona and Biokinetics of Cerium Oxide Nanoparticles

Nagarjun Konduru, Ramon M Molina. Harvard T.H. Chan School of Public Health, USA.

How do physicochemical properties of nanoparticles (NPs) influence their biokinetics and biological outcomes? We assessed the effects of an amorphous silica coating on the pharmacokinetics and biological effects of CeO_2 NPs. Uncoated and silica-coated CeO_2 NPs with primary NP size of 33 nm were generated by flame spray pyrolysis and later neutron-activated. These radioactive NPs were IT-instilled, gavaged, or intravenously injected in rats. Animals were analyzed over 28 days post-IT, over 7 days post-gavage and 2 days post-injection. Our data indicate that silica coating caused more but transient lung inflammation compared to uncoated CeO_2. The transient inflammation of silica-coated CeO_2 was accompanied by its enhanced clearance. Then, from 7 to 28 days, clearance was similar although significantly more 141Ce from silica-coated (35%) was cleared than from uncoated (19%) $141CeO_2$ in 28 days. The protein coronas of the two NPs were significantly different when they were incubated with either alveolar lining fluid or plasma. Despite more rapid clearance from the lungs, the extrapulmonary ^{141}Ce from silica-coated $^{141}CeO_2$ was still minimal (<1%) although lower than from uncoated $^{41}CeO_2$ NPs. Post-gavage, nearly 100% of both NPs were excreted in the feces consistent with very low gut absorption. Both IV-injected $^{141}CeO_2$ NP types were primarily retained in the liver and spleen. The silica coating significantly enhanced the retention of ^{141}Ce in other organs except the liver. We conclude that silica coating of nanoceria alters the biodistribution of cerium likely due to modifications in the protein corona formation.

Keywords: Biocorona, Biodistribution, Biokinetics/toxicokinetics, Cerium oxide nanoparticles

41. Green Toxicology Meets Nanotoxicology: the Process of Sustainable Nanomaterial Development and Use

Harald F. Krug. Empa, Switzerland.

Chemistry has influenced our lives during the last century as Nanotechnology will do during the 21st century. We had in the past a story-book full of severe accidents and tragedies triggered by malpractice and misbehavior with chemicals [1]. At the end of the 20th century Nicholas Anastas published a book on the principles of Green Chemistry [2], new rules which should help to prevent such accidents or misuse of chemicals. Four of the 12 principles of Green Chemistry deal with toxicology. The actual situation in nanotechnological material development and nanotoxicology is somehow disappointing, as we started early with the accompanying safety research on nanomaterials but we didn't respect the principles of good laboratory practice and Green Chemistry/Green Toxicology. Thus, we have an overwhelming amount of studies demonstrating biological effects in vivo or in vitro but most of these studies are not suitable for risk assessment or regulatory purposes [3]. The consequence from this fact must be the introduction of the idea of Green Toxicology into the risk research on nanomaterials. Respecting the 7 principles of Green Toxicology [4] will help to achieve the goal of safer nanomaterials as has been shown recently [5].

[1] European Environment Agency (2001) P. Harremoes et al. (eds.), 22, Copenhagen; [2] P.T. Anastas, J.C. Warner (1998) Green Chemistry. Oxford University Press, New York; [3] H.F. Krug (2014) Angew. Chem. Int. Ed. 53, 12304-12319; [4] A. Maertens et al. (2014) ALTEX. 31:243-249; [5] T. Walser, C. Studer (2015): Regul. Toxicol. Pharmacol. 72, 569-571.

Keywords: Green nanomaterials, Sustainability, Green Chemistry, Green Toxicology

42. Safety Assessment of Nanomaterials Using the ECETOC DF4nano Decision-Making Framework

Robert Landsiedel[1], Josje H.E. Arts[2], Athena Keene[3], Reinhard Kreiling[4], Delina Lyon[5], Monika Maier[6], Karin Michel[7], Thomas Petry[8], Ursula G. Sauer[1], David Warheit[9], Karin Wiench[1], Wendel Wohlleben[1]. [1]BASF SE; [4]Clariant Cosmetics; [6]Evonik; Henkel[7]; [8]Toxminds, Germany; [2]AkzoNobel, Netherlands; [3]Afton Chemical; [9]Chemours, USA; [5]Shell Oil Company, UK.

The European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) 'Nano Task Force' proposes a Decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping) consisting of 3 tiers to assign nanomaterials to 4 main groups (with possible further sub-grouping) and to refine specific information needs. The DF4nanoGrouping covers all relevant aspects of a nanomaterial's life cycle and biological pathways: intrinsic material and system-dependent properties, biopersistence, uptake and biodistribution, cellular and apical toxic effects. Use, release and exposure route may be applied as 'qualifiers' to determine if, e.g., nanomaterials cannot be released from products, which may justify waiving of testing. The four main groups encompass (1) soluble, (2) biopersistent high aspect ratio, (3) passive, and (4) active nanomaterials. The DF4nanoGrouping foresees a stepwise evaluation of nanomaterial properties and effects with increasing biological complexity: As necessary, intrinsic material properties, system-dependent properties (e.g. surface reactivity and dispersibility that are dependent upon the nanomaterial's respective surroundings, such as culture media or lung lining fluid) and in vitro effects are assessed. In vitro cytotoxicity testing (preferably using alveolar macrophages as relevant in vitro test system for inhalation exposure) plays a central role in determining nanomaterial functionalities. The DF4nanoGrouping facilitates grouping and targeted testing of nanomaterials. It ensures that sufficient data for grouping and ultimately risk assessment of a nanomaterial are available, and it fosters the use of non-animal methods. At the same time, no studies are performed that do not provide crucial data. Thereby, the DF4nanoGrouping serves to save both animals and resources. COI: Some of the authors are employees of companies producing and marketing nanomaterials.

Keywords: Hazard ranking/characterization, In vitro toxicology, In vivo toxicology, Regulatory decision making

43. Long-Term Inhalation Study with Nanomaterials: Effects and Lung-Burden after Chronic Inhalation Study with Ceria and Barium Sulfate

Robert Landsiedel[1], Lan Ma-Hock[1], Jana Keller[1], Sibylle Groeters[1], Tom Gebel[2], Dirk Schaudien[3], Peter Laux[4], Karin Wiench[1]. [1]BASF SE; [2]BAuA; [3]Fraunhofer ITEM; [4]BfR, Germany.

Lung carcinogenicity and putative systemic effects of low-dose life-time inhalation exposure to biopersistent nanoparticles were examined in a chronic inhalation study performed according to OECD test guideline no. 453 with several protocol extensions. Female rats (100/group) were exposed to cerium dioxide (NM-212, 0.1; 0.3; 1; 3 mg/m³) and barium sulfate (NM-220; 50 mg/m³) for two years; a control group was exposed to clean air. Lung burdens and burdens in extrahepatic tissues were measured at various time-point. The two year exposure period was successfully terminated and 50 animals per dose group were examined for organ burden and histopathology. The remaining animals currently are kept exposure-free for maximally 6 additional months. Up to two years exposure to both nanoparticles did not lead to body weight reduction compared to control animals. The mortality rates were in an acceptable range. Macroscopically evident tumors were not detected after two years. The CeO_2 lung burdens were maximally 3.5 mg/g lung tissue at the highest exposure concentration of 3 mg/m³. In comparison, highest CeO_2 burdens in organs remote to exposure were liver and spleen with maximally roughly 1 x 10^{-3} g/g tissue. In brain, maximum CeO_2 levels were $7x10^{-6}$ mg/g lung tissue. $BaSO_4$ lung burdens were comparatively low (1 mg/g) within the first 13 weeks of exposure and steeply increased to 6 mg/g lung tissue after one year. The comprehensive histopathological examinations of lungs and other tissues will be finalized in 2017. COI: RL, LMH, JK and SG are employees of BASF - the company is producing and marketing nanomaterials.

Keywords: In vivo toxicology, Inhalation

44. New Developments in Understanding Nanomaterial Dose

Jamie Lead. University of South Carolina, USA.

The fundamental dose-response relationship in (eco)toxicology is complicated when considering nanomaterials because of uncertainties around dose measurement. Complications arise because of issues such as choosing the correct dose-metrics, nanomaterial transformations in exposure media and organisms, and our ability to accurately assess exposure and dose concentrations. All of these aspects have led to a situation, where nanomaterials properties, transformations and exposure and dose measurement are generally still largely ignored in the field, despite repeated discussions at conferences, review papers and other sources. This talk will discuss two developments: 1) the development of a four-layered, isotopically-labelled core-shell nanomaterial (nanohybrid) which can be used to distinguish exposure and dose from the nanomaterials and from ions (from nanomaterial dissolution); 2) developments in single particle ICP-MS including conceptual nanomaterial separation, use in complex media and measurement of nanomaterial number concentration within cells.

Keywords: Dosimetry, Environmental nanotoxicology

45. Cerium Dioxide Nanoparticles Modulate Bleomycin Induced Inflammatory and Fibrotic Events within the Rat Lung

Martin Leonard, Chang Guo, Alison Buckley, Sarah Robertson, Kirsty Meldrum, James Warren, Alan Hodgson, Timothy Gant, Rachel Smith. Public Health England, UK.

The use of cerium dioxide nanoparticles (CeO_2NP) in diesel fuel catalysts and other applications has led to concerns over health effects in situations of inadvertent exposure. In an attempt to understand how CeO_2NP may influence oxidative stress induced pulmonary inflammation and fibrotic events we used an in vivo bleomycin model of lung injury. Male Sprague-Dawley rats were intra-tracheal instilled with bleomycin or saline followed by nose-only inhalation exposure to aerosolised CeO_2NP (mass concentration 1.8 mg/m^3, primary particle size 5 – 10 nm, aerosol count median diameter 40 nm) or water (controls) for 3 hours per day, 4 days per week for one or two weeks. Three days post exposure, animals were sacrificed and analysed for lactate dehydrogenase levels and cell counts in bronchoalveolar lavage (BAL) fluid. Lung histopathology was also examined using masons trichrome staining. Analysis of global mRNA expression changes was carried out using an RNA-Seq poly(A) library based sequencing method. Bleomycin exposure resulted in an increase in total BAL cells, LDH and fibrotic staining at 1 and 2 weeks of exposure together with a significant induction of inflammatory and extracellular matrix regulators on sequencing analysis. Modifications of these responses by CeO_2NP exposure included attenuation of fibrotic staining and gene expression markers of lung function, inflammation and ECM turnover. There was however no significant alterations in total BAL cell count. CeO_2NP treatment alone resulted in increased inflammatory responses, BAL cell counts and inflammatory gene expression and tissue injury but did not appear to cause fibrotic changes within the lung.

Keywords: Cerium oxide nanoparticles, Hazard ranking/characterization, In vivo toxicology, Systems biology/toxicology

46. Engineered Nanoparticles in Food Matrices and their Behavior and Toxicity in the Gastrointestinal Tract

Mengshi Lin, Azlin Mustapha. University of Missouri, USA.

There has been growing interest in recent years in introducing nanoscale additives and ingredients into food matrices. In addition, foods can be contaminated by engineered nanomaterials from packaging materials, nano-sized pesticides, or other sources. This has raised some concerns for consumers. There remains a need to detect and investigate the behavior and toxicity of engineered nanoparticles (ENPs), and to provide critical information about the safety of ENPs in food matrices. The objectives of this project were to extract, detect, and characterize ENPs in food matrices by a combination of techniques; study the behavior and transformation of ENPs in the gastrointestinal (GI) tract using in vitro gastric and intestinal models; study the potential effects of ENPs on the natural gut microflora (E. coli, L. acidophilus, B. animalis), and investigate the toxicity of NPs on Caco-2 cell lines. Three types of ENPs were selected in this study, including selenium, silver, and zinc oxide nanoparticles.

Keywords: Emerging nanomaterials, In vitro toxicology, Metal/metal oxide nanomaterials, Methods

47. PBPK Modeling of Gold Nanoparticles: A Tool to Extrapolate from Animals to Man

Zhoumeng Lin[1], Nancy A. Monteiro-Riviere[2], Raghuraman Kannan[3], Jim E. Riviere[1]. [1]Institute of Computational Comparative Medicine and [2]Nanotechnology Innovation Center of Kansas State, Kansas State University; [3]Department of Radiology, University of Missouri, USA.

Gold nanoparticles (AuNP) can be used as diagnostic, therapeutic agents, and drug delivery systems. Interspecies and in vitro to in vivo extrapolation of pharmacokinetic and toxicity data is crucial for successful translation from laboratory studies to humans to aid risk assessment of AuNP. This study formulated a physiologically based pharmacokinetic (PBPK) model of AuNP for animal-to-human extrapolation. This model described endocytosis of PEG-coated AuNP of different size ranges (13-20 nm; 80-100 nm) in major phagocytic organs of mice. The mouse model was subsequently refined and extrapolated to rats and pigs for AuNP with different coatings; and then extrapolated from mice, rats or pigs to humans. The derived human model was used to incorporate available toxicity data to estimate human equivalent doses. These results showed that endocytosis of AuNP was time- and size-dependent; i.e. endocytosis of larger PEG-coated AuNP occurred immediately and predominately from the blood, whereas smaller PEG-coated AuNP could diffuse through the capillary wall and their endocytosis appeared mainly from the tissue with a 10-h delay. This may be the primary mechanism for the reported size-dependent pharmacokinetics of AuNP. Animal-to-human extrapolation results showed that rats and pigs seemed to be better models than mice, and that dose and age should be considered in this extrapolation. The human equivalent dose associated with reported cytotoxicity concentration (13 µg/ml) in human dermal fibroblasts was 1mg/kg. This PBPK model provides insights into size-dependence and animal-to-human extrapolation of AuNP pharmacokinetics; this methodology may also be applied to other nanomaterials with proper calibration. Funding provided by the Kansas Bioscience Authority.

Keywords: Biokinetics/toxicokinetics, Dosimetry, Metal/metal oxide nanomaterials, PBPK modeling

48. Phenotypic Differences in Aortic and Microvascular Endothelial Cells Influences Responses to Cerium Dioxide Nanoparticles

Valerie Minarchick[1], Jonathan H Shannahan[1], Edward M Sabolsky[2], Jared M Brown[1]. [1]University of Colorado Denver; [2]West Virginia University, USA.

Exposure to cerium dioxide nanoparticles (CeO_2) has been shown to cause vascular dysfunction in animal models. However the mechanisms and target cell types of vascular dysfunction are unknown. We hypothesized that phenotypic differences in aortic and microvascular endothelial cells will influence responses to CeO_2 exposure and ultimately impact vascular dysfunction. Rat aortic endothelial cells (RAEC) or rat microvascular endothelial cells (RMEC) were exposed to CeO_2 (0-100 µg/ml). Cytotoxicity, alterations in inflammatory (IL-6) and surface adhesion marker (ICAM and VCAM) mRNA expression, and reactive oxygen species (ROS) generation were assessed following exposure. To assess potential influences on vascular function, isolated naïve arterioles were intraluminally exposed to the supernatant from CeO_2 exposed RAEC and RMEC. Endothelium-dependent and –independent reactivity was assessed with acetylcholine (ACh, 10-9-10-4 M), and spermine NONOate (10-9-10-4 M). Finally, enhanced darkfield microscopy and flow cytometry was used to determine CeO_2 cellular uptake in vitro. Following a 3 hr exposure to 25 µg/ml CeO_2, RAEC had an increase in nitric oxide synthase (NOS, 2.5-fold) and VCAM (3.3-fold) mRNA expression. RMEC also had an increased VCAM expression (2.8-fold), which was similar to the RAEC; however, NOS mRNA expression (35.7-fold) in RMEC was significantly higher than the RAEC. Lastly, CeO_2 exposure did not elicit ROS production in RAEC but was increased 23±1.3% in RMECs as compared to control following 25 µg/ml treatment. Taken together, these results indicate that the aortic and microvascular endothelium respond uniquely to CeO_2. Furthermore, these distinct responses may have differential impacts on vascular function. R01-ES019311 (JMB), K99-ES024392 (JHS).

Keywords: Cerium oxide nanoparticles, In vitro toxicology, Toxicological mechanisms

49. Kinetic Model Structures and Limitations Imposed by Idiosyncratic Infusion-Related Reactions to Nanoparticles

Seyed Moein Moghimi. Durham University, UK.

Pharmacokinetics and biological performance of intravenously injected nanopharmaceuticals are controlled by a complex array of interrelated core and interfacial physicochemical and biological factors. Definitive maps that establish the interdependency of nanoparticle size, shape and surface characteristics in relation to biodistribution, controlled drug release, excretion, and adverse effects are still poorly outlined. This in turn poses a grand challenge to the physiologically based pharmacokinetics modelling of nanopharmaceuticals. Within the context of nanomedicine-mediated infusion-related cardiopulmonary distress a role for the complement system activation has been proposed. In many individuals nanoparticle administration incites complement, but only some patients undergo cardiopulmonary distress. Pigs are often used as predictive models of nanomedicine-mediated infusion-related reactions in humans, but there is no evidence to refute infusion reactions in pigs may proceed independently of complement activation. Pulmonary intravascular macrophages (PIM), however, are abundant in pig lungs. Robust phagocytosis of particles by PIM results in immediate release of large quantities of mediators that correlate with periods of peak cardiopulmonary disturbances, which can be blocked by indomethacin administration. Human lung lacks PIM, but there are suggestions of induction of pulmonary macrophages in certain human diseases. It is conceivable that highly responsive patients may have induced PIM, which could increase sensitivity to blood-borne particles, and the potential risk of pulmonary hemodynamic side effects. This presentation critically examines the interplay between induced PIM, the complement system, and the potential risk of cardiopulmonary side effects on nanomedicine administration. Alternative and improved models will be suggested as well as strategies for safer nanoparticle engineering.

Keywords: In vitro toxicology, In vivo toxicology, Toxicological mechanisms, Nanomedicine

50. Pulmonary Distribution of Nanoceria: Comparison of Intratracheal Bolus and Microspray Instillation and Dry Powder Insufflation

Ramon Molina, Joseph D. Brain. Harvard T.H. Chan School of Public Health, USA.

Nanoparticles (NPs) may be delivered to the respiratory tract of laboratory animals using various techniques. Inhalation exposure is more physiological but leads to variable deposited dose, requires large amounts of aerosolized NPs and includes nasopharyngeal and fur NP deposition. Although less physiological, intratracheal (IT) instillation of suspended NPs allows for precise dosing (small or large), and a specified time zero. Insufflation can deliver NPs in their native dry form as an aerosol. We compared the distribution of neutron-activated 141CeO2 NPs (5mg/kg) in rats after 1) IT instillation, 2) microspraying of a nanoceria liquid suspension and 3) insufflation. Blood, tracheobronchial lymph nodes, liver, the gastrointestinal tract, feces and urine were collected 24 hours post-dosing. Lungs from each rat were removed and were dried at room temperature inflated at a constant 30 cm water pressure. Dried lungs were then sliced into 50 pieces.Radioactivity of each piece was measured. The evenness index (El) of each piece was calculated [EI=(μCi/mgpiece)/(μCi/mglung)]. The degree of departure from 1.0 is a measure of the deposition heterogeneity. We showed that the pulmonary distribution of deposited nanoceria differs with each mode of administration. Dosing using IT or microspraying resulted in similar spatial distribution. Insufflation of dry powder resulted in significant deposition in the trachea and in more heterogeneous lung distribution. The greatest heterogeneity in distribution was with dry powder insufflation. We conclude that animal dosing techniques and devices result in different doses and pattern of particle deposition that may impact the results of biokinetic and toxicity results.

Keywords: Biodistribution, Biokinetics/toxicokinetics, Cerium oxide nanoparticles

51. Use of Predictive Approaches in EHS Regulation

Jeffery Morris. US EPA, USA.

Twenty-first century testing approaches, including high-throughput assays, are going to play an increasing role in chemical evaluation by the US EPA under the Toxic Substances Control Act, including for nanomaterials. As with traditional testing approaches, understanding what properties affect toxicity is important to understanding the extent to which test results can be generalized beyond the specific test substance. High-throughput approaches have the potential to more-quickly and cost-effectively test a large number of variants of particular nanomaterial types (for example, changes in particle coatings or particle functionalization). However, the ability to run the assays on variants assumes knowledge of whether a variation in a nanoparticle changes key properties of the particle and if so, whether such changes will affect assay results. These approaches also are going to be an increasingly important part of developing chemical categories; for example, to enable the ability to “read across” existing data on multiwalled carbon nanotubes (CNT) to infer environmental behavior to structurally similar CNTs for which data are not available.

Keywords: Carbon-based nanomaterials, Environmental nanotoxicology, Hazard ranking/characterization

High throughput screening

52. Trophic Transfer and Bioaccumulation of Carbon Nanotubes in a Microbial Food Chain

Monika Mortimer[1], Elijah J. Petersen[2], Bruce A. Buchholz[3], Eduardo Orias[1], Patricia A. Holden[1]. [1]University of California, Santa Barbara; [2]National Institute of Standards and Technology; [3]Lawrence Livermore National Laboratory, USA.

Carbon nanotubes (CNTs) can enter the environment through many applications, for example via agriculture and consumer products. Sustainable development of CNT-enabled products requires efficient assessment of potential hazards of CNTs, including their propensity to bioaccumulate and biomagnify. Here, we used ^{14}C -labeled multiwall carbon nanotubes (^{14}C-MWCNTs) at low CNT concentrations (0.004–1 mg/L) estimated to occur in the environment to study CNT accumulation and trophic transfer in a microbial food chain comprised of the bacteria Pseudomonas aeruginosa and the protozoan Tetrahymena thermophila. Working with such low concentrations of ^{14}C -MWCNTs was enabled by the novel application of accelerator mass spectrometry (AMS). Both bacteria and protozoans accumulated considerable amounts of CNTs, by absorption to cell surfaces and by phagocytosis into the food vacuoles, respectively, when exposed to CNTs directly in aqueous media. When protozoans preyed on CNT-encrusted bacteria, CNTs were trophically transferred and bioaccumulated in protozoans, although to a lesser extent than via direct exposure to CNTs. Logarithm-transformed bioconcentration factors [LOG(BCF)] of CNTs in bacteria ranged from 5.1to 5.4. Bioaccumulation factors [LOG(BAF)] in protozoans were 3.1 to 3.5 and 2.9 for direct exposures and trophic transfer, espectively. These values indicate the high propensity of CNTs to adsorb to bacterial cells and accumulate in protozoans, likely making CNTs bioavailable for organisms at higher trophic levels. The study was funded by NSF and EPA under Cooperative Agreements DBI-1266377 and DBI-0830117, NIH/NIGMS 8P41GM103483, the trust of Mr. Henry H. Wheeler, Jr., Estonian Research Council grant PUTJD16 and US DOE LLNL Contract DE-AC52-07NA27344.

Keywords: Carbon-based nanomaterials, Environmental nanotoxicology, Exposure assessment, trophic transfer, bioaccumulation, bioconcentration, biomagnification

53. Use of Alternative Test Strategies and AOP-Based High Content Screening for Predictive Hazard Profiling and Tiered Risk Assessment

Andre Nel. California Nano Systems Inst. at University of California Los Angeles, USA.

My talk will outline the nano EHS infrastructure that we have developed for predictive toxicological assessment of engineered nanomaterials (ENM) to allow high content screening of nanomaterial libraries based on adverse outcome pathways (AOPs) involved in the pathophysiology of disease. I will describe the establishment of predictive platforms and structure-activity relationships (SARs) for a number of ENMs categories to indicate how the hierarchical ranking of ENMs can be used for tiered decision analysis that can be deployed for regulatory purposes, and safer design of nanomaterials. Our studies will delineate how the use of a predictive paradigm for carbon nanotubes can be used for tiered risk assessment analysis, which can be used in combination with exposure data in a decision tree aimed at limiting the volume of testing that is needed for every new material being introduced, as well as for the purposes of ENM categorization. We will demonstrate how a variety of CNT libraries were used to delineate the importance of tube number, the influence of surface coating, surface functionalization, chirality chirality and catalytic activity, as well as how CNTs compare to graphene and graphene oxide toxicity. I will discuss how the lessons learned for CNTs can be used preemptively to address future safety concerns that may arise during the widespread implementation of this category of carbonaceous materials in the marketplace. I also discuss the pitfalls of the use of ATS and how to strengthen this investigative platform in future work.

Keywords: Adverse outcomes pathway analysis

54. Emerging Metrology for High Throughput Nanomaterial Genotoxicology

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The rapid development of the engineered nanomaterial (ENM) manufacturing industry has accelerated the incorporation of ENMs into a wide variety of consumer products across the globe. It is thus prudent to have rapid and robust analytical metrology in place that can be used to critically assess and/or predict the cytotoxicity, as well as the potential genotoxicity of these ENMs. NIST has established methodology based upon hyphenated/tandem mass spectrometry (GC/MS/MS and LC/MS/MS) for the measurement of ENM-induced genotoxicity. These methods are highly sensitive and allow absolute identification and quantification of base or nucleoside damage induced by exposure to ENMs. However, these methods suffer from very low sample throughput. Recently, a number of sensitive, high throughput genotoxicity assays/platforms (CometChip assay, flow cytometry/micronucleus assay, flow cytometry/g-H2AX assay, automated "Fluorimetric Detection of Alkaline DNA Unwinding" (FADU) assay, ToxTracker reporter assay) have been developed, based on substantial modifications and enhancements of traditional genotoxicity assays. These assays have been used for the rapid measurement of DNA damage (strand breaks), chromosomal damage (micronuclei) and for detecting upregulated DNA damage signalling pathways resulting from ENM exposures. I will briefly describe the fundamental measurement principles and measurement endpoints of these new assays, as well as their modes of operation, analytical metrics and potential interferences, as applicable to ENM exposures. A description of the major technical advantages and limitations of each assay for evaluating and predicting the genotoxic potential of ENMs will also be given.

Keywords: Genotoxicity, High throughput screening, In vitro toxicology

55. An EH&S Approach for Commercialization of Novel Forms of Nanocellulose

Kimberly Nelson. American Process Inc., USA.

American Process Inc. (API) is the world's first company to produce and market six different varieties of nanocellulose products with tailored morphologies and surface properties to enhance the performance of a wide variety of materials across various industries. API's BioPlus™ nanocellulose products are manufactured using a low cost, patented process that is demonstrated at their fully-integrated Thomaston Biorefinery plant along with lignocellulosic sugars, fuels and chemicals co-products. This presentation will discuss our EH&S approach, including toxicity testing, for commercialization of a variety of novel forms of nanocellulose. Nanocellulose is a versatile material with a vast array of commercial applications including composites and foams for automotive, aerospace, and building construction, viscosity modifiers for cosmetics and oil drilling fluids, and high performance fillers for paper, packaging, paints, and plastics. Nanocellulose is renewable, biodegradable, biocompatible and as strong as Kevlar™. Reuter's recently named nanocellulose as one of ten innovations that will transform the world by 2025. The US USDA estimates that global production of nanocellulose could reach 34 million tons per year in the coming decades.

Keywords: Commercialization, Emerging nanomaterials

56. Use of Life Cycle Analysis in Responsible Design of a Quantum Dot Display Product and Manufacturing Process

Robert Nick. QD Vision, Inc., USA.

In the design of quantum dot (QD) based optical products for the display industry, life cycle risk assessment identified a number of areas in which environmental, health and safety risks could be mitigated. Energy savings afforded by the use of QD optics with high quantum efficiencies yielded high gamut displays with considerable reduction in energy utilization. The more efficient displays yielded a net reduction in carbon foot print of the product as well as a savings in emitted cadmium in excess of the amount used in the product. Specific matrix design of the product also ensured that the QDs would be immobile at the end of the product life cycle with no release of Cd containing nanoparticles detected in TCLP tests of the optics. Additionally, manufacturing process changes were made which eliminated the use of pyrophoric QD precursors from the process and significantly decreased the quantities and risk severity of the solvents used in this process.

Keywords: Commercialization, Emerging nanomaterials, quantum dots

57. Concepts of Dosimetric Modeling of Rodent Inhalation Studies with Inhaled Nanoparticles: The Need for Allometric Scaling of Respiratory Parameters for Extrapolation to Humans

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Analyzing Exposure-Dose-Response relationships of inhaled nanoparticles (NPs) for purposes of risk assessment requires detailed information about physicochemical properties, airborne behavior of the particles, species-specific respiratory tract geometry, and breathing parameters. The widely-used Multiple-Path Particle Dosimetry (MPPD) model was developed as a tool to predict deposition and also retention of aerosolized particulate materials in the respiratory tract of various mammalian species. NP aerosol characteristics as well as default or bodyweight-adjusted values for breathing parameters and lung volumes can be entered. Default values, though, can give rise to inaccurate predictions for the modeled output deposition fractions. To prevent this, we replaced the default values with bodyweight-adjusted respiratory parameters, published either specifically for the rat or as allometric power functions developed for mammals. Regarding particle characteristics, MPPD requires the aerosol density as input. Because of the void spaces of aerosolized agglomerated NPs, the effective density is significantly lower than the material density. To refine the input for density, the deposited lung dose in rats from a single whole-body inhalation study needs to be measured for obtaining the deposition fraction. Running the MPPD model and adjusting the density to result in the same deposition fraction defines the effective density for the specific aerosol. The result can subsequently be applied to determine mechanical and dissolution clearance rates of biopersistent and biosoluble NPs in longer-term inhalation exposure studies. The validity of this approach as well as its applicability to dosimetric extrapolation modeling of results from rodent studies to a Human Equivalent Concentration will be demonstrated.

Keywords: In silico modeling, extrapolation modeling

58. Overcoming the Endosomal Escape Problem: Lysosome-Targeting Gold Nanostar Nanoconstructs

Teri W. Odom. Northwestern University, USA.

A perceived disadvantage of nanoparticle-based therapeutics is the inability of the nanoconstructs to escape from vesicles and thus induce a biological response. This talk will describe how this challenge can be overcome while keeping in mind a nanoconstruct design that can reduce potential cytotoxic effects. Gold nanostar particles are advantageous as nano-carriers because they are anisotropic in shape, facile carriers of bio functional ligands, and prepared using bio-compatible precursors. We select aptamers (chemical antibodies) as therapeutic and targeting ligands to reduce unwanted immune responses and to simplify scalability of the nanoconstruct. As a model system, we focused on targeting HER2 as the well-known biomarker and transmembrane protein in breast cancer cells and gold nanostar constructs based on anti-HER2 aptamers grafted to gold nanostars (HApt-AuNS). We found that HApt-AuNS showed improved delivery and anti-cancer effects in targeted cancer cells over free HApt. In order to correlate progression in the endocytosis process with cellular response, we monitored the sub-cellular localization of HER2-HApt-AuNS complexes using confocal fluorescence microscopy and differential interference contrast microscopy. At the same time points, we measured accumulation of HER2-HApt-AuNS complexes in lysosomes, lysosomal activity, and lysosomal degradation of HER2 and discovered that all were positively correlated. Therefore, by both targeting lysosomes and exploiting degradation of HER2 on accumulated HER2-HApt-AuNS within lysosomes, we can begin to solve the endosomal escape problem that is a challenge for all nanoparticles.

Keywords: Emerging nanomaterials, Metal/metal oxide nanomaterials

59. Searching for Risk, Finding Value: Unexpected Developments from Nanotoxicology/Industry Collaborations

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[1]nanoComposix; [2]Food and Drug Administration; [3]Air Force Research Laboratory; [4]Kansas State University, USA.

Over the last 10 years, nanotoxicology research has uncovered a surprising depth of complexity in how nanoparticles interact with biological systems. Subtle changes in size, shape and surface result in unexpected protein interactions, changes to transport and differences in cell uptake. With a goal of developing quantitative structure activity relationships, toxicologist have developed a deep data set of nano-bio interactions that provide a foundation for producing safer nano-enabled products and future nanomedical devices and therapies. Commercial case studies will be presented that depict how robust, reproducible nanomaterials developed in collaboration with nanotoxicologists and safer-by-design principles are accelerating nanotechnology commercialization. COI: nanoComposix supplies nanoparticles to the nanotoxicology research community

Keywords: Commercialization, Metal/metal oxide nanomaterials, Physicochemical characterization

60. Functional nanotoxicology applied to crop species exposed to engineered nanomaterials

Luca Pagano[1], Alia D. Servin[2], Roberto De la Torre-Roche[2], Sanghamitra Majumdar[2], Joseph Hawthorne[2], Nelson Marmiroli[3], Om Parkash Dhankher[1], Jason C. White[2], Marta Marmiroli[3], Elena Maestri[3]. [1]University of Massachusetts; [2]The Connecticut Agricultural Experiment Station, USA; [3]University of Parma, Italy.

In vivo functional toxicology is already utilized for the identification of genes involved in tolerance and sensitivity to nanomaterial exposure in model plant systems such as Arabidopsis. Appropriate biomarkers can be used as both as descriptors and predictors of exposure and effects. Following ortholog identification (metabolic functions, stress response, transport, protein synthesis, DNA repair) across different species, transcriptomics can significantly augment morphological and physiological data on plant response. The aim of the project was to identify sensitive molecular biomarkers in agricultural crops (tomato, zucchini) that not only indicate exposure to engineered nano materials (ENM; CuO, CeO_2, La_2O_3) but also provide a mechanistic understanding of the response. Plant response (up-/down-regulated genes) varied by both particle type, size and species. Importantly, some of the targets found constitute consistent biomarkers of nanomaterial exposure hat may be useful for assessing ENM risk. In a separate trophic transfer study, lettuce was grown in soil 0-70d after amendment with CuO NP, bulk and ions (0-400mg/kg); crickets and lizards were used as first and secondary consumers. Expression levels of genes involved in $Cu2^+$ transport in lettuce suggest that mechanisms involved in CuO bulk and NPs response are different from that of Cu ions. The significance of these findings and the utility of the functional approach will also be discussed.

Keywords: Environmental nanotoxicology, Metal/metal oxide nanomaterials, Risk assessment, Toxicological mechanisms

61. Biomolecular Effects of TiO_2 Exposures in Humans: What did Nanotoxicology Tell Us?

Daniela Pelclova[1], Vladimir Zdimal[2], Petr Kacer[3], Nadezda Zikova[2], Martin Komarc[1], Zdenka Fenclova[1], Stepanka Vlckova[1], Jaroslav Schwarz[2], Otakar Makeš[2], Kamila Syslova[3], Tomas Navratil[4], Francesco Turci[5], Ingrid Corazzari[5], Sergey Zakharov[1], Jaroslav Belacek[1], Dhimiter Bello[6]. [1]Charles University in Prague; [2]Institute of Chemical Process Fundamentals of the AS CR, v.v.i.; [3]Institute of Chemical Technology Prague; [4]J. Heyrovský Institute of Physical Chemistry of the CAS, Czech Republic; [5]Department of Chemistry, University of Torino, Italy; [6]University of Massachusetts, Lowell, Work Environment Department, College of Health Sciences, USA.

Nanoscale titanium dioxide (nanoTiO_2) is a commercially important engineered nanomaterial. Animal studies have documented lung injury and inflammation, oxidative stress, cytotoxicity and genotoxicity. Yet human health data are extremely scarce and quantitative risk assessments and biomonitoring of exposure are lacking. A study was carried out with 34 workers exposed to (nano) TiO_2 pigment and 45 controls. In their exhaled breath condensate (EBC) titanium and markers of oxidation of nucleic acids (8-hydroxy-2-deoxyguanosine, 8-hydroxyguanosine, 5-hydroxymethyl uracil); proteins (o-tyrosine, 3-chlorotyrosine, and 3-nitrotyrosine), lipids (malondialdehyde, 4-hydroxy-trans-hexenale, 4-hydroxy-trans-nonenale, 8-isoProstaglandin F2α and aldehydes C6-C12,), and leukotrienes B4, C4, E4, and D4 were analyzed. Spirometry and fractional exhaled nitric oxide (FeNO) were measured. In the workshops, the median total mass 2012 and 2013 TiO_2 concentrations were 0.65 and 0.40 mg/m^3, respectively. The median number concentrations measured by the scanning mobility particle sizer (SMPS) and aerodynamic particle sizer (APS) were $1.98x10^4$ and 2.32x104 particles/cm^3, respectively. About 80% of those particles were smaller than 100 nm in diameter. Titanium in EBC was significantly higher, and all oxidative stress and inflammation markers in EBC were higher in production workers relative to the controls ($p<0.01$), including the pre-shift EBC samples. Multiple regression analysis confirmed an association between the production of TiO_2 and the levels of studied biomarkers. Spirometry and FeNO were not sensitive and/or specific enough to reveal impairments. Accordingly to experimental findings, these results are consistent with the oxidative stress hypothesis and suggest, at the molecular level, sustained lung injury.
Acknowledgements: P25/1LF/2, P28/1LF/6.

Keywords: Human toxicology, Metal/metal oxide nanomaterials, Occupational safety

62. Advances in 3-Dimensional In Vitro Models as Well as with In Vivo Approaches for Genotoxicity Testing of Nanomaterials

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A number of nanomaterials have been shown to interfere with genome integrity using standard in vitro OECD guideline genotoxicity assays. When extrapolating from ‘hazard’ to ‘risk’, a critical limitation of these 2-dimensional methods are A) their inability to model pharmacokinetic/toxicokinetic characteristics relevant to the human exposure scenario in question, and B) their inability to mimic, or explore, the mode of action (MoA) that may lead to relevant genotoxicity risk. For example, human skin will usually provide a strong penetration barrier towards particulate materials and this important, exposure-limiting feature is missing in standard cell culture-based methods. These often use very high doses that can lead to spurious findings irrelevant to the corresponding risk scenario - here dermal exposure. The availability of standardized human cell-based 3-dimensional models now allows for exposure scenarios to be addressed more directly, providing higher relevance towards potential human risk. The second main limitation of standard OECD genotoxicity assays is their inability to distinguish primary DNA damaging effects from effects that occur secondary to high levels of cytotoxicity or indirect tri ggers like oxidative stress. To this end, modified in vivo OECD methodologies can provide important insight into the MoA triggering a certain DNA-damaging effect, with huge impact on risk assessment. The presented example will show results from in vivo Comet and micronucleus assays with nano silica materials clearly demonstrating that the observed genotoxicity effects are triggered by local tissue inflammation rather than by direct, DNA damaging properties of the particles which should enable setting of safe exposure limits.

Keywords: Genotoxicity, Hazard ranking/characterization, Risk assessment, Toxicological mechanisms

63. Engineered Nanoparticles Emitted From Laser Printers: A Case Study Of Environmental Health Implications From Nano-Enabled Products During Consumer Use

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Laser printers has been associated with emission of particulate matter (PM), ozone, volatile organic compounds and most recently, engineered nanomaterials (ENMs). Thus, a multi-tiered methodology was designed to physico-chemically, morphologically and toxicologically characterize the ENMs released from nano-enabled toners during printing. An exposure system was developed and used to screen commercially available laser printers. It was confirmed that toner formulations contained and released ENMs released to the air during printing. Some printers can release ENMs at concentrations higher than a million particles/cm^3. These Printer-Emitted Particles (PEPs) have complex chemical composition, primarily of organic nature, and include metal/metal oxides. Furthermore, the biological responses to PEPs were evaluated using cellular and animal experimental models. Both models showed PEPs are biologically reactive and may cause significant cytotoxicity, membrane integrity damage, oxidative damage, pro-inflammatory cytokine release, angiogenesis, cytoskeletal, epigenetic changes and overall lung inflammation. These results suggest that PEPs are chemically complex and bioactive and thus, may be deleterious to individuals exposed in residential and occupational settings. More importantly, we demonstrated the importance of understanding environmental health and safety implications of nano-enabled products across their life-cycle and assessing real world exposures to the released PM rather than the ‘‘raw’’ ENMs used in their synthesis. This methodology across the exposure-toxicology continuum can be used for toxicological assessment of other nano-enabled products.

Keywords: Environmental nanotoxicology, In vitro toxicology, In vivo toxicology, Life cycle analysis

Nanomaterial release

64. Multi-Walled Carbon Nanotube Physicochemical Properties Predict Pulmonary Inflammation and Genotoxicity

Sarah Søs Poulsen[1], Kirsten Kling[1], Kristina B. Knudsen[1], Vidar Skaug[2], Zdenka O. Kyjovska[1], Birthe L. Thomsen[1], Per A. Clausen[1], Rambabu Atluri[1], Stefan Bengtson[1], Henrik Wolff[3], Keld A. Jensen[1], Håkan Wallin[1], Ulla Vogel[1]. [1]The National Research Centre for the Working Environment, Denmark; [2]National Institute of Occupational Health, Norway; [3]Finnish Institute of Occupational Health, Finland.

Lung deposition of multi-walled carbon nanotubes (MWCNT) induces pulmonary toxicity. However, because of their great inter-variance in physicochemical properties, the exact drivers of MWCNT toxicity are yet to be determined. To identify determinants of toxicity, we analyzed effects of 10 commercial MWCNT (supplied in three groups of different dimensions, with one pristine and two/three surface modified in each group). We characterized morphology, chemical composition, surface area, and functionalization. MWCNT were deposited in lungs of female C57BL/6J mice by intratracheal instillation of 0, 6, 18 or 54 μg/animal. Pulmonary inflammation (neutrophil influx in bronchoalveolar lavage (BAL)) and genotoxicity were determined at 1, 28 or 92 days, and histopathology of the lungs at 28 and 92 days. Effects were related to dose, time and physicochemical properties. Inflammation persisted on day 92 for the thin (14-17 nm) MWCNT. Histologically, lymphocytic aggregates were detected with all MWCNT on post-exposure day 28 and 92. Using adjusted, multiple regression analyses, specific surface area (BET) was identified as a predictor of increased pulmonary inflammation on all post-exposure days. In addition, length significantly predicted pulmonary inflammation, whereas surface oxidation (-OH and -COOH) was predictor of lowered inflammation on day 28. BET surface rea, and therefor diameter size, significantly predicted genotoxicity in BAL fluid cells and lung tissue. Our approach of defining a grouping principle by toxicological effects may contribute to safe-by-design-MWCNT, for minimizing adverse effects. The project was supported by Danish Centre for Nanosafety, grant# 20110092173-3 from the Danish Working Environment Research Foundation.

Keywords: Carbon-based nanomaterials, Genotoxicity, In vivo toxicology, Physicochemical characterization

65. Emerging Two-Dimensional Materials: Potential Health Risks and Benefits

Apparao M. Rao. Clemson University, USA.

Two-dimensional (2D) materials are a new class of materials that are one to a few atomic layers in thickness (e.g., graphene, graphene oxide, MoS2, h-BN), which exhibit valuable but complex properties for use in electronics, medicine, energy generation and storage. For instance, semiconducting transition metal dichalcogenides such as MoS2 exhibit a band gap that is presently being exploited for photo-thermal therapy and drug delivery while graphene and BN are being explored as excellent coatings for heart valves and stents. The mass production of such nanomaterials naturally raise questions related to their physiological response, for example: i) how are the nano-bio interactions of emerging 2D materials distinct from their 0D and 1D counterparts? and ii) could their adverse interactions be mitigated through innovative material design? Our recent findings suggest that amino acids and proteins exhibit different binding mechanisms with 2D materials in which the charge transfer from proteins plays a vital role. More importantly, we showed that the charge transfer from proteins can be mitigated in case of single-layer graphene due to its unique electronic structure. Despite such recent progress, many issues on physicochemical characterization of 2D materials continue to plague the interdisciplinary field of nanotoxicity. This talk will focus on critical characteristics of 2D materials that lead to an altered physiological response warranting new theoretical and experimental studies to understand in detail their biological interactions at the molecular level.

Keywords: Emerging nanomaterials

66. Nanoparticle Coating and Genetic Make-Up of the Host Modulate Genotoxicity of Ingested Silver Nanoparticles

Ramune Reliene, Sameera Nallanthighal. State University of New York at Albany, USA.

Incorporation of silver nanoparticles (AgNPs) into food packaging materials, dental care products and dietary supplements may result in oral exposure to AgNPs. We examined whether oral exposure to AgNPs cause DNA damage and genomic instability in vivo in mice. We found that sub-acute ingestion of 4 mg/kg of citrate-coated-AgNPs (cit-AgNPs) induced oxidative DNA damage and double strand breaks in peripheral blood, chromosomal damage in bone marrow and DNA deletions in developing embryos. In contrast, PVP-coated-AgNPs (PVP-AgNPs) were not genotoxic. Moreover, cit-AgNP-induced damage persisted for at least 14 days after treatment termination. Because oxidative damage and strand breaks are repaired rapidly after their formation, their presence after treatment cessation indicates that cit-AgNPs persist in the body. Our mechanistic studies showed that cit-AgNPs downregulated base excision repair (BER) genes that repair oxidative DNA lesions. We hypothesized that AgNPs induce oxidative DNA damage and consequently genomic instability via downregulation of BER genes, which predicts that BER defects enhance sensitivity to AgNPs. We tested this hypothesis in mice deficient in Ogg1, a BER enzyme that excises oxidized guanine. Ogg1 deficient mice were hypersensitive to AgNP-induced oxidative and chromosomal damage. In summary, these data demonstrate that ingestion of cit-AgNPs at a dose equivalent to 800x oral RfD for daily exposure to silver induces DNA damage and permanent genome alterations and may therefore cause cancer. In addition, our data suggest that Ogg1 polymorphisms and mutations, which are common in humans, may enhance susceptibility to AgNP-mediated genome damage. Funding provided by NIH/NIEHS R56 ESO24123 to RR.

Keywords: Carcinogenicity, Genotoxicity, In vivo toxicology, Metal/metal oxide nanomaterials, Susceptibility

Toxicological mechanisms, silver nanoparticles

67. Physiological Based Pharmacokinetic (PBPK) Modeling of Nanomaterials: Why is Quantitation and Anatomical / Physiological Reality So Important?

Jim Riviere. Kansas State University, USA.

As the field of nanotoxicology advances, numerous studies using in vitro and in vivo animal models are conducted across a wide variety of nanomaterials with the goal of predicting biological activity in humans. In addition to the obvious differences in biology across species, differences in anatomical and physiological properties of organs and organisms dictate certain factors that must be accounted for when trying to make reasonable predictions in humans. These include the physicochemical properties of tissue fluids and blood using in vitro versus in vivo models that may modulate protein corona formation and composition, the mismatch of time scales between both metabolic processes across species with purely chemical processes, as well as specific biological differences in cells, membranes and organs across species. In order for data to be properly extrapolated across species and levels of model systems, an approach is required which enables the integrating of these results in a quantitative and statistically valid framework. Physiological based pharmacokinetic (PBPK) models, widely used for small molecule drug development and chemical risk assessment, are ideally suited to serve this purpose. This presentation will review the philosophy and background of PBPK models applied to small organic molecules and overview the unique processes involved in nanomaterial biodistribution that must be accounted for. In addition, how PBPK models may be constructed and adapted for nanomaterial biodistribution will be discussed. This presentation will lay the foundation for additional speakers in this symposium to illustrate specific nanomaterial PBPK models.

Keywords: Biokinetics/toxicokinetics, Biokinetics/Toxicokinetics, Biodistribution, In silico modeling

68. Nanoparticle Dosimetry to the Point - Air-Liquid Exposure Systems to Deliver an Accurate Dose onto the Lung Cell Surface In Vitro

Barbara Rothen-Rutishauser. Adolphe Merkle Institute, University of Fribourg, Switzerland.

Because the lung is considered by far the most important portal of entry into the human body for aerosolized nanomaterials released into the environment (during production, processing, or intended usage of products), much research focuses on this organ as a potential barrier. Although both epidemiologic and animal studies are often carried out, many studies to investigate the potential adverse effects of inhaled nanomaterials are performed using lung cell cultures in vitro. These studies take into consideration the efforts by national and international authorities to reduce, refine and replace (3R) animal experiments with in vitro approaches to investigate toxic effects on human cells in extensive, mechanistic studies. Air-liquid exposure scenarios can mimic nanomaterial exposure in the lung and several different systems have been developed for the deposition of spherical particles as well as high aspect ratio materials, i.e. cellulose whiskers or carbon nanotubes, at the air-liquid interface of cultured lung cells. The advantage of this system is that we can fully control the material characteristics and also monitor the mass deposition on the lung cell surface on-line, allowing us to determine a dose-effect correlation. An overview will be given about the advantages and limitations of such systems but also how they can be combined with sophisticated 3D multicellular tissue models that allow the investigation of possible effects of nanomaterials under more realistic conditions.

Keywords: Alternative testing methods/strategies

69. The effect of topically applied carbon nanomaterial on skin inflammation in a model of allergic Bio-Distribution and Toxicity of Orally Delived PLGA Nanoparticles, Effect of Charge

Cristina Sabliov[1,2], Sara Navarro[2], Timothy Morgan[3], Rhett Stout[1], Diana Coulon[2], Carlos Astete[2]. [1]Louisianna State University; [2]College of Agriculture, Louisianna State University; [3]Michigan State University, USA.

Over the past few decades, polymeric nanoparticles have gained scientific interest because of their potential medical applications as drug delivery systems. The aim of this project was to quantify the biodistribution and to assess the toxicity of PLGA (poly-lactic-co-glycolic acid) and surface modified PLGA chitosan (PLGA/Chi) nanoparticles orally administered for 7, 14 and 21 days to F344 rats. Fluorescent nanoparticles were tracked in F344 rat tissues, and toxicity was evaluated by alkaline phosphatase (ALP) and alanine transaminase (ALT) levels, and by histologic examination of tissue samples. Biodistribution of PLGA and PLGA/Chi were similar, with highest amounts found in the intestine and liver. ALT increased significantly in treated rats. Mild histological differences were detected in the intestine and liver. PLGA and PLGA/Chi nanoparticles behaved similarly presenting minimal toxicity in the liver and intestine, but not in kidney, lung and brain.

Keywords: Biodistribution, In vivo toxicology

70. Head-to-Head Comparison of In Vitro Cytotoxicity and Phytotoxicity of Antimicrobial Mixed-Valence Cu Nanocomposites with Commercially Available Cu Pesticides

Swadeshmukul Santra, Mikaeel Young, Parthiban Rajasekaran. University of Central Florida, USA.

Copper (Cu) bactericides/fungicides are aggressively used in the agriculture industry. There is an increasing concern on Cu accumulation in fertile soil and for it's leaching potential into the surrounding ecosystem. Moreover, development of bacterial Cu resistance is a serious concern. While there is no suitable alternative to Cu available to date for agricultural use; strategies for improving efficacy through engineering of Cu valence states, materials structure and its environment are attractive options. To address these limitations, we developed mixed valence (MV) Cu loaded composites. It is hypothesized that MV Cu system, specifically enriched with Cu (0) and Cu(I) will exhibit enhanced antimicrobial efficacy over traditional Cu (II) compounds. MV-Cu served as a combination of both soluble and insoluble Cu compounds when silica/chitosan matrix used as delivery system. Materials were characterized by HRTEM, XPS, XRD and AAS for size, crystallinity and composition. Plant safety results showed that our Cu nanocomposites were non-phytotoxic and have comparable rainfastness against industry control Kocide 3000. Preliminary in-vitro antimicrobial efficacy of the MV-Cu was evaluated a number of model plant bacterial species, Xanthomonas alfalfae subsp. citrumelonis, Pseudomonas syringae pv. syringae and Clavibacter michiganensis subsp. michiganensis showing improved efficacy over the industry standards. In my talk, I will focus primarily on toxicity assessment of the Cu based nano-composite bactericides using lung and macrophage model systems.

Keywords: Environmental nanotoxicology, Copper, citrus, tomato, citrus canker, bacterial spot, nanotechnology

71. Occupational Safety and Health: Green Chemistry Sustainability and Regulatory Policy Issues in Green Nanotechnology

Paul Schulte, Laura Hodson, Mark Hoover, Charles Geraci. National Institute for Occupational Safety and Health, USA.

Nanotechnology presents an opportunity to develop new approaches and industrial applications in a sustainable way from the outset. By identifying, assessing, and pursuing risk-informed pathways and policies to define and apply green nanotechnology, it should be possible to move beyond the legacy infrastructures and approaches that characterize traditional production efforts. The key to making that possibility a reality, however, will be the development and use of a risk-informed framework. In this presentation the risk assessment paradigm of hazard identification, exposure assessment, risk assessment/characterization and risk management will be explored in terms of the driving questions: what do we know, what do we need to know, and how can we obtain needed knowledge. This 3 x 4 framework provides for twelve cells that taken together will address the occupational safety and health issues of green nanotechnology. Clearly hazard identification is a critical enabling feature of this framework. To date, toxicology studies have made progress in hazard identification but a critical need is for better material characterization which impacts the whole risk assessment paradigm. While hazard identification is important, workers, employers, and authorities need information on all aspects of the risk assessment continuum simultaneously since development of green nanotechnology is already occurring.

Keywords: Green nanomaterials, Occupational safety, Sustainability

72. The Benefits and Applications of Cerium Oxide Nanoparticles

William Self, Atul Dhall, Sudipta Seal, Soumen Das. University of Central Florida, USA.

The development of metal oxide nanomaterials as industrial catalysts has brought great promise for improved catalysis driven by material science research. Whether more reactive catalysts at the nanoscale may also yield materials with higher toxicity in biological systems remains a concern. Cerium oxide nanoparticles (CeNPs) have shown promise both as an industrial catalyst and a biological biomaterial, yet some studies have revealed apparent toxicity in both plant and mammalian cell culture and in vivo model systems. In contrast many studies show a protective effect of CeNPs in cell culture and model in vivo systems. The core reactive properties that these nanomaterials exhibit, superoxide dismutase mimetic activity, catalase mimetic activity, and general radical scavenging properties, are still poorly understood at the molecular level. A number of in vitro and in vivo studies continue to support a positive biological outcome when CeNPs are introduced into inflammatory models in which reactive oxygen and nitrogen species are a critical node, yet contradictory studies in toxicology are hard to reconcile. Ultimately it is likely that the method of synthesis, route of delivery and the concentration of nanomaterial is ultimately the key parameters to these seemingly contradictory findings. This report will summarize the key positive biological studies to date and try and resolve the debate on the toxicity that has been reported. An emphasis on recent work to describe the active site of catalysis of CeNPs, coupled with a historical view of their impact on life science research will be the focus of this work.

Keywords: Biomedical/therapeutic applications, Cerium oxide nanoparticles, Emerging nanomaterials, Metal/metal oxide nanomaterials

73. Disease-Induced Modification in the Identity of the Nanoparticle-Biocorona and Toxicity

Jonathan Shannahan[1], Jared M Brown[2]. [1]Purdue University, College of Health and Human Sciences; [2]University of Colorado Anschutz Medical Campus, USA.

Nanoparticles (NPs) associate macromolecules forming a biocorona (BC) when introduced into biological systems, altering properties and toxicity. The BC is dependent on NP physicochemical properties and the physiological environment. Individuals with obesity and cardiovascular disease exist with altered physiological environments, which may influence NP toxicity. We hypothesize that a BC formed on NPs following incubation in hyperlipidemic serum will result in altered NP-BC protein content, cellular uptake, and toxicity compared to normal conditions. We utilized rat aortic endothelial cells (RAEC) and 20 nm Fe_3O_4 NPs that are being developed for biomedical applications. A BC was formed on Fe_3O_4 NPs following incubation in 10% normal or hyperlipidemic serum. Addition of BCs resulted in increased hydrodynamic size and decreased zeta potential. Fe_3O_4 NPs associated more cholesterol following incubation in hyperlipidemic serum. We identified differences in BC protein components via LC-MS. To assess BC-induced differences in uptake, RAEC were exposed for 2h to 10 μg/ml of Fe_3O_4 NPs, Fe_3O_4 NPs with normal or hyperlipidemic BCs and analyzed by ICP-MS and dark-field microscopy. Fe_3O_4 NPs without a BC were readily internalized by RAEC however addition of BCs reduced uptake. To evaluate BC-induced differences in cell activation an endothelial cell specific PCR array was utilized. For a number of genes including IL-6, TNF-α, Cxcl-2, VCAM-1, ICAM-1, and Selectin-E addition of the BCs was found to exacerbate RAEC responses to Fe_3O_4 NPs (Fe_3O_4 -Hyperlipidemic > Fe_3O_4 -Normal Fe_3O_4). These findings demonstrate the possible influence of disease-induced variations in physiological environments and their impact on NP toxicity. Funding K99 ES024392

Keywords: Biocorona, In vitro toxicology, Metal/metal oxide nanomaterials

74. Neuroprotection and Neurotoxicity of Nanoparticles in the Central Nervous System with Special Reference to Nanomedicine

Hari Shanker Sharma[1], Dafin F. Muresanu[2], Jose Vicente Lafuente[3], Z. Ryan Tian[4], Asya Ozkizilcik[4], Ranjana Patnaik[5], Aruna Sharma[1]. [1]Uppsala University, Sweden; [2]Department of Clinical Neurosciences, University of Medicine & Pharmacy, Cluj-Napoca, Romania; [3]Department of Neurosciences, University of Basque Country, Spain; [4]Department of Chemistry & Biochemistry, University of Arkansas, Fayetteville, USA; [5]Indian Institute of Technology, Banaras Hindu University, India.

Nanoformulation enhances neurotherapeutic values of drugs or neurodiagnostic tools as compared to their parent compounds. However, effects of nanoparticles per se in inducing neurotoxicology, if any is still being largely ignored. Thus, role of nanomedicine is to enhance the drug availability within the central nervous system (CNS) for greater therapeutic successes. However, once the drug-nanoparticle complexes enter into the CNS, the fate of nanomaterial is largely unknown. Thus, to achieve greater successes in nanomedicine expanding our understanding of nanoneurotoxicology is the need of the hour. In our studies, we observed that intoxication of nanoparticles e.g., Ag, Au, Cu, Al, SiO_2, single walled carbon nanotubes (SWCNTs) administered systemically in rats or mice induces neurotoxicity e.g., disruption of the blood-brain barrier (BBB), development of brain edema and neuronal, glial, axonal and endothelial cell damages. Furthermore, when additional traumatic brain or spinal cord injuries inflicted in these animals the magnitude of brain pathology was enhanced by 150 to 300 % depending on the type of nanoparticles used. Ag, Cu and SiO_2 nanoparticles exhibited the most marked exacerbation of brain pathology following injury. In such situations, neuroprotective agents if given either in double doses or administered through nontechnology, e.g., TiO_2 nanowired delivery achieved better neuroprotection than the parent compound given alone. These observations clearly indicate that nanomedicine is the need of the hour to induce clinical benefits in situation with co-morbidity factors e.g., nanoparticles exposure after central nervous system (CNS) injury.

Keywords: Biomedical/therapeutic applications, neurotoxicity, nanodrug delivery

75. Alternative Testing Strategies for Categorizing Nanomaterials in Regulatory Decision Making

Jo Anne Shatkin[1], Kimberly Ong[2]. [1]Vireo Advisors, USA; [2]Lund University, Sweden.

There have been significant advancements in the development and use of alternative testing strategies (ATS) for assessing the toxicology and biological mechanisms of action for chemicals and more recently, for nanomaterials. There is significant interest and pressure to adopt ATS in the reduction, refinement and replacement of animal testing in a regulatory context, particularly for use in categorizing novel materials. Yet, the adoption of new methods for regulatory testing is a measured process that can take years to achieve validation and acceptance. This talk will frame the issues for regulatory decision making and discuss recommendations from a Society for Risk Analysis/ OECD Working Party on Manufactured Nanomaterials pilot project focused on the state of the science and opportunities for the use of ATS in categorizing novel nanomaterials for risk assessment.

Keywords: Alternative testing methods/strategies, Commercialization, Environmental nanotoxicology, Exposure characterization, Regulatory decision making, Risk assessment

76. Nanocellulose Green Natural Products: Toxicology Prospective

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The development of renewable polymeric materials have received great public attention due to the growing demand for environmental friendly products. Nanocellulose, a unique and promising natural material, has gained interest for its broad use as a reinforcement in the manufacturing of high performance polymer composites, building materials, cosmetics, food, and drug industry. Because of its remarkable physical features, special surface chemistry and excellent biological properties (e.g. biocompatibility, biodegradability and perception of low toxicity), CNC products have already hit the market. Cellulose nanocrystals (CNC) is a crystalline form of cellulose obtained from different sources including wood pulp. High aspect ratio and stiffness of CNC may cause similar pulmonary toxicity as carbon nanotubes and asbestos, thus posing a negative impact on public health and the environment. The present study was undertaken to investigate the pulmonary outcomes induced by exposure to respirable CNC. Pulmonary inflammation/damage, accelerated oxidative stress, impaired pulmonary functions, cytogenic alterations seen by frequency of micro-nucleated cells in BAL fluids, and increase of TGF-b and collagen in the lungs was discovered after exposure to CNC. Notably, these effects were significantly more pronounced in female mice compared to male. Moreover, gender differences in responses to pulmonary exposure to CNC were also detected at the level of global RNA expression as well as in cytokine/chemokine responses. Overall, our results clearly indicated that exposure to respirable CNC caused sustained pulmonary injury.

Keywords: Green Nanomaterials, Green Nanomaterials, Gender Differences, Cellulose, Pulmonary Exposure, Pulmonary Injury

77. Quantitative Analysis of the Physicochemical Properties of Cerium Oxide Nanomaterials and their Influence on Nano-Bio Interactions

Christopher Sims, Justin Gorham, Russell Maier, Alline Myers, Aaron Johnston-Peck, Shannon Hanna, Igor Levin, Vincent Hackley, Bryant Nelson. National Institute of Standards and Technology, USA.

Of the many engineered nanomaterials being incorporated into our society, cerium oxide (CeO2-x, ceria) nanoparticles (NPs) are receiving increased attention due to their unique chemical properties and vast number of current and potential applications (e.g. automotive catalysts, UV filters, agricultural treatment agents, antioxidant therapeutics, etc.). As the overall environmental and toxicological outcomes of ceria NPs are not yet fully understood, it is imperative to develop a comprehensive understanding of their physicochemical properties since these properties will influence the interactions of ceria NPs with biological and environmental systems. Here, we utilize multiple analytical techniques to thoroughly characterize a suite of ceria NPs, both as dry powders and in environmentally relevant media. Highlighted are the use of X-ray photoelectron spectroscopy (XPS), electron energy loss spectroscopy (EELS), and low-angle annular dark field scanning transmission electron microscopy (LAADF-STEM) to orthogonally characterize the oxidation states of the ceria NPs. Our results suggest the primary particle size has a large effect on the oxidation state of the ceria NPs, with smaller particles having increased Ce^{3+}/Ce^{4+} ratios compared to their larger counterparts, in agreement with previous research. Additional experiments suggest the identity and amount of surface coating on the ceria NPs highly impact their resultant dynamics in biologically-relevant media. Preliminary results of toxicity assays using the environmentally relevant model organism, Caenorhabditis elegans (C. elegans) will also be presented, with discussion of how the physicochemical characteristics of ceria NPs could influence the uptake, distribution, and potential mechanisms of toxicity in biological and environmental systems.

Keywords: Cerium oxide nanoparticles, Environmental nanotoxicology, In vivo toxicology, Physicochemical characterization

78. Nano-Waste: Environmental Health and Safety Implications During Thermal Degradation/ Incineration of Nano-Enabled Products at Their End-Of-Life

Dilpreet Singh[1], Georgios Sotiriou, Fang Zhang[1], Joey Mead[2], Dhimiter Bello[2], Wendel Wohlleben[3], Philip Demokritou[1]. [1]Center for Nanotechnology and Nanotoxicology, Harvard T.H. Chan School of Public Health; [2]Center for High-Rate Nanomanufacturing, University of Massachusetts-Lowell, USA; [3]BASF SE, Germany.

Conventional nanotoxicology has addressed adverse effects of pristine ENMs but little is known about their property-transformation or Particulate Matter release over the life-cycle (called LCPM) of a nano-enabled product (NEP). End-of-life of NEPs poses a significant nano-waste problem since nearly 20,000 tons of ENMs end up in incinerators every year globally. We assessed thermal decomposition of industrially-relevant thermoplastic nanocomposites, using a versatile Integrated-Exposure-Generation-System (INEXS), that allows for systematic investigation of physicochemical, morphological and toxicological properties of decomposition byproducts (LCPM, residual ash) under various combustion conditions.Various polymer matrices (PU, PE, PP, PC, EVA) enabled with different nanofillers (CNT, carbon black, Fe_2O_3, organic dye, TiO_2) were investigated at nano-loadings(0.115wt%) at decomposition temperatures: 500, 8000C. Matrix type affected CNT release as PP showed nearly 8 times higher release than PC at 5000C. Organic carbon in LCPM was >99wt% across all matrices and nanofiller loadings; however, both matrix type and nanofiller loading influenced the PAH composition and possibly the toxicological profile of released LCPM.Size and morphology of LCPM were mainly governed by matrix type,whereas morphology of residual ash was strongly influenced by bulk presence of nanofiller. Effect of increasing titania loading (2-15wt%) on titania release wasn't evident because of very minimal release (<0.02 wt%). However, increasing CNT loading from 0.1wt% in PU to 3 wt% in PP and PC resulted in quantifiable amount of CNT release in air from PP (0.3 wt%) and PC (0.04wt%) compared to non-detectable release from PU. Findings also raise EHS concerns regarding LCPM exposure in incineration facilities and ENM release from residual ash into environmental media following disposal. Addressing these EHS concerns will help formulate a novel nano-risk assessment paradigm based on realistic life-cycle exposures and nanotoxicology of LCPM.

Keywords: Environmental nanotoxicology, Exposure characterization, Life cycle analysis, Nanomaterial release, nano-enabled products, thermal decomposition, environmental health and safety

79. Inhalation Toxicity of 5 – 10 nm Cerium Dioxide Nanoparticles

Rachel Smith[1], Chang Guo[1], Alison Buckley[1], Martin Leonard[1], Ralf Weber[2], Sarah Robertson[1], Tim Marczylo[1], Kirsty Meldrum[1], James Warren[1], Alan Hodgson[1], Mark Viant[2]. [1]Public Health England; [2]University of Birmingham, UK.

Cerium dioxide nanoparticles (CeO_2NPs) are used in a number of diesel fuel additives to improve fuel combustion efficiency and exhaust filter operation, and they have been detected in ambient air in locations where such additives are in use. Concerns have been raised about the potential impacts on human health of inhaling CeO_2NPs and an in vivo inhalation study has been undertaken to investigate. Male Sprague-Dawley rats were exposed in a nose-only inhalation exposure system to aerosolised CeO_2NPs (mass concentration 1.8 mg/m^3, primary particle size 5 – 10 nm, aerosol count median diameter 40 nm) or water (controls) for 3 hours per day for 4 days per week for one or two weeks. Animals were sacrificed at 3 and 7 days post-exposure. Bronchoalveolar lavage was undertaken and samples of lung and other tissues generated. Analysis of total and differential cell type counts, LDH, TP, AP and cytokine release in bronchoalveolar lavage fluid, and lung histopathology indicates an inflammatory response greater than that seen in other studies, emphasising the importance of particle size. Laser Ablation ICP-MS imaging indicates the presence of cerium in terminal bronchioles and alveolar spaces and in kidney and liver sections. Tissue samples have been analysed using synchrotron X-ray spectroscopy to investigate the location and speciation (XANES) of the cerium, as this is considered a key toxicity driver. The above results and those of the ongoing gene expression and metabolomics analysis of lung tissue will be presented. This study was funded by the UK NERC.

Keywords: Biokinetics/toxicokinetics, Cerium oxide nanoparticles, In vivo toxicology

80. Gestational Nanomaterial Exposure: A Cardiovascular Perspective

Phoebe Stapleton, Carroll McBride, Jinghai Yi, Timothy Nurkiewicz. West Virginia University, USA.

Engineered nanomaterial (ENM) inhalation has been shown to lead to a variety of cardiovascular consequences in young healthy male models of exposure. The continued development of ENM has given rise to concerns over the potential for human health effects. Given our interest in cardiovascular endpoints, we elected to evaluate ENM exposure in one of the most complex and acutely demanding circulations, the maternal uterine microvascular adaptations that support fetal development. Starting after implantation (gestational day [GD] 6) pregnant Sprague-Dawley rats were exposed to nano-titanium dioxide aerosols for 4 days per week through GD 20 (count mode aerodynamic diameter of 151 ± 2.2 nm, 11.3 ± 0.2 mg/m^3, 4 hrs/day) to evaluate the maternal and fetal consequences of ENM inhalation. The calculated daily maternal deposition was 15±0.3 µg. We have shown microvascular endothelial reactivity in exposed animals to be significantly blunted than controls at each level of the materno-fetal system (maternal, fetal, and adult progeny). Maternal endothelial dysfunction has been identified using in vivo (-58%±22) and in vitro (-42%±10) preparations. Endothelial dysfunction persists in the tail artery of the fetal pup (-30%±11) on gestational day 20. These microvascular impairments are widespread and maintained into adulthood, with endothelium-dependent dysfunction evident in the coronary (-137%±35) and uterine circulations (-71%±12). These results provide evidence of significant microvascular dysfunction at each level of development after ENM exposure during gestation: maternal, fetal, and maintained into adulthood. The cardiovascular implications associated with gestational ENM exposures are widely understudied. NIH-K99-ES024783 (PAS); NIH-R01-ES015022 (TRN).

Keywords: Developmental nanotoxicology, Systems biology/toxicology, gestation, cardiovascular

81. Fine-Tuning Properties of Carbon Nanomaterials for Biomedical Applications

Alexander Star, University of Pittsburgh, USA.

Single-walled carbon nanotubes, and more recently graphene, have attracted considerable interest for the development of chemical sensors. These carbon nanostructures are just one atom thick, thus making their electronic properties extremely sensitive to adsorption of chemical species on their surface. When decorated with metal or metal oxide nanoparticles, these nanostructures can exhibit a large and selective electronic response toward many analytes, creating potential applications in medical diagnostics. Carbon nanostructures can also be used as nano-carriers for applications in medical therapeutics. To this end, we have developed cup-shaped carbon nanostructures, which have an elliptical hollow space that can be corked by gold nanoparticles at the opening. To illustrate the potential of corked nanocups as drug delivery systems, loading with common fluorescent dyes, as well as chemotherapeutic drugs, was performed. By using Raman spectroscopy, we were able to demonstrate the loading and release of the cargo in biochemical model systems and tumor-associated cells.

Keywords: Carbon-based nanomaterials, Emerging nanomaterials, drug delivery

82. The Biocorona: Lessons Learned and Challenges Accepted?

Roland H. Stauber, Dana Westmeier, Dominic Docter. Molecular and Cellular Oncology, University Hospital of Mainz, Germany.

Whereas the physico-chemical properties and behaviour of nanoparticles (NPs) can be characterized accurately under idealized conditions, this is no longer the case in complex physiological or natural environments. Here, NPs adsorb biomolecules upon contact with all biological environments. Therefore, biomolecules-coated NPs may need to be considered as ‚new materials' compared to the pristine NPs during their manufacturing. Particularly, the so called 'NP-protein corona' not only critically impacts nanotoxicology and nanoecology but also influences the success and safety of nanomedical applications. Hence, a mechanistic understanding of its relevance and the mechanism regulating corona formation is mandatory. Based on recent insights, we here critically discuss concepts of corona formation and evolution. We comment on how corona signatures may be linked to effects at the nano-bio interface including cellular uptake and biodistribution. We conclude how NP material and/or surface modifications may be rationally exploited to shape the protein corona in order to rationally improve their safety. We introduce the impact of the corona for NM-microbiome-(human)host interactions and conclude by discussing relevant challenges, which need to be resolved by the field. Own References: Feliu, et al. 2016. Chem Soc Rev. 10.1039/c5cs00699f. Docter, et al. 2015. Chem. Soc. Rev. 44:6094 - 6121. Tenzer, S., et al. 2013. Nature Nanotechnol. 8:772-781. Funding: BMBF DENANA, NanoBEL; ChemBioMed.

Keywords: Biocorona

83. Nanoparticle Exposure of Persistently Herpesvirus-Infected Lung Cells Reactivates Latent Virus and Restores Features of an Acute Virus Infection In Vitro and In Vivo

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Both inhalation of ambient nanoparticles (NP) as well as persistent herpesvirus-infection have been implicated to contribute to the development of chronic lung disease. To question whether NP exposure during a latent virus infection may disrupt the anti-viral immune control and induce virus reactivation, we conducted in vitro studies with latently infected cell lines, and in vivo studies with mice, using the murine gammaherpesvirus 68 (MHV-68) model system. Our results show that Exposure of latently infected murine or human cells with spherical carbon nanoparticles (CNP) or double-walled carbon nanotubes (CNT) induced lytic virus production and expression of the viral transactivators Rta and BZLF1. In the lungs of latently infected mice (28 days after infection), immunohistochemistry demonstrated an increase in lytic viral proteins 24h after exposure to CNP or CNT, a response usually observed during the acute lytic phase (6 days after infection). Likewise gene expression analysis of lung homogenates revealed a pro-inflammatory signature with considerable parallels the pattern seen during the acute lytic phase. Analysis of the lung metabolome demonstrated an enrichment of phospholipids in the lungs of latently infected mice after exposure to CNP, matching profoundly with the pattern observed during acute virus infection. Taken together, our results indicate that the combination of NP exposure and persistent herpesvirus infection restores a molecular signature found in acute virus infection, boosts production of lytic viral proteins, and induces an inflammatory response in the lung – a pattern which might finally result in severe tissue damage and even fibrotic alterations.

Keywords: Adverse outcomes pathway analysis, Carbon-based nanomaterials, In vivo toxicology, viral infection

84. Emerging Biosensing Applications of Carbon Nanotubes and Graphene with Implications for Mitigating Nanotoxicology by Design

Michael S. Strano. Massachusetts Institute of Technology, USA.

Our lab has been interested in how the 1D/2D electronic structures of carbon nanotubes and graphene respectively can be utilized to advance new concepts in molecular detection, including sensors. We introduce CoPhMoRe or corona phase molecular recognition as a method of discovering what in practice are synthetic antibodies, or nanotube-templated recognition sites from a heteropolymer library. We show that certain synthetic heteropolymers, once constrained onto a single-walled carbon nanotube by chemical adsorption, also form a new corona phase that exhibits highly selective recognition for specific molecules. Several examples of heteropolymers–nanotube recognition complexes for riboflavin, L-thyroxine, dopamine, nitric oxide, sugar alcohols, estradiol, as well as proteins such as fibrinogen will be presented. An emerging application of such sensor technology has been the use of near infrared fluorescent carbon nanotube sensors for in-vivo detection of a variety of biomolecules. Here, we show that PEG-ligated d(AAAT)7 DNA wrapped SWNT are selective for nitric oxide, a vasodilator of blood vessels, and can be tail vein injected into mice and localized within the viable mouse liver. We use an SJL mouse model to study liver inflammation in vivo using the spatially and spectrally resolved nIR signature of the localized SWNT sensors. We also demonstrate that a hydrogel encapsulated version of the sensor can remain viable and biocompatable in A129 mice for more than 400 days, opening the possibility of long term, persistent biochemical detection through thick tissue. Current projects include glucose, insulin and cortisol sensing in-vivo, and applications to other analytes will be discussed.

Keywords: Carbon-based nanomaterials, Emerging nanomaterials

85. Adverse Outcome Pathways: A Framework for Organizing Mechanistic Information to Improve Toxicity Assessment

Kristie Sullivan. Physicians Committee for Responsible Medicine, USA.

For a variety of reasons, there is interest in moving away from whole animal toxicology testing and towards a more human-relevant, predictive approach. The Adverse Outcome Pathway (AOP) concept has gained popularity because it can help to put existing information, and especially mechanistic information, into biological and regulatory context. An AOP is a linear framework that links an initial molecular interaction with a chemical, via a series of essential and testable “key” events at various levels of biological organization, to an adverse outcome, often of regulatory interest. The framework is meant to be flexible and adaptable to the dataset it organizes, and need not be complete or detailed in order to be useful. An AOP’s suitability for different uses is dependent on the confidence that can be assigned via a weight of evidence evaluation of the available evidence it contains. Recognition of the concept’s utility has led to a global, OECD-led, crowd-sourced effort to create a network of AOPs using known toxicological and biological information. AOPs can then be used to support read-across, QSAR, test guideline, and testing strategy use and development. In specific fields of research, AOPs can organize streams of research data from in vitro, in vivo, and epidemiological studies in order to generate testable hypotheses, highlight mechanistic research gaps, and identify tools to characterize the toxicity of nanomaterials alone or relative to other nano or bulk chemicals.

Keywords: Adverse outcomes pathway analysis, Systems biology/toxicology

86. Benign-By-Design: The Nexus between Green Chemistry, Toxicology, and Nano-Material Science

Joel Tickner[1], Molly Jacobs[2]. [1]Department of Community Health and Sustainability; [2]Lowell Center for Sustainable Production, University of Massachusetts, Lowell, USA.

While significant efforts have been made to improve the health and safety of engineered nano-materials, their development has occurred at a significantly faster rate than our ability to understand impacts. Yet, increasing scientific evidence provides reason to be concerned about potential worker health, consumer, and end of life risks. The development of nanomaterials, with the focus primarily on cost and performance, parallels the development of most industrial chemicals. The field of green chemistry provides an opportunity to integrate considerations of health and safety at the design phase of materials, so that they are considered equal to cost and performance. This presentation outlines some of the potential unintended consequences we may expect to see by developing engineering nanomaterials without adequate consideration of health, safety and environment. We outline a number of approaches and new collaborations aimed at bringing together the fields of green chemistry and toxicology to more effectively design materials that are not only high performing and cost-effective but also health protective. We conclude by presenting principles and design guidelines for designing safer nanomaterials.

Keywords: Occupational safety, Regulatory decision making, Risk assessment

87. In Vitro Aerosol Exposure System for Nanotoxicity: Dosimetric Analysis using Nanometal Oxides

Trevor Tilly, Tyler Nelson. Air Force Research Labs, USA.

Determining the delivered cellular dose of nanomaterials (NMs) is critical for Nanotoxicology studies. It is often difficult to make comparisons between published studies due to the multitudes of natural and man-made NMs and their solubility issues combined with the difficulty in creating a monodispersed liquid dispersion. Additionally, there is a need for more enhanced in vitro methods that evaluate the toxicity of NM deposited at the air-liquid interface of the respiratory tract. Aerosol exposure chambers (AECs) have been developed to expose NMs to cell cultures at the air-liquid interface; however, dosimetry of NMs using these chambers requires improvement. Currently, most studies published provide a determination of dose estimated prior to the exposure using electron microscopy and gravimetric analysis techniques; however, these approaches do not take in account the complex nature of aerosols and the inconsistency of deposition makes determining the actual respiratory system regional delivered dose quite difficult. In this study, we dispersed nanopowders of ZnO, CeO_2, NiO, and CuO in air and exposed these individually to lung cells using our AEC design. Cellular viability and inflammation (IL-8) were determined at 24 hrs after exposures. Dosimetry was predicted using the Fusch and Gunn theory of aerosol data collected from the optical and scanning mobility particle sizers, and the delivered dose was determined using inductively coupled mass spectrometry. High doses of NMs decreased cellular viability, whereas significant induction of the inflammatory marker IL-8 was observed 24 hrs after low level exposure to CuO, ZnO, and NiO NMs.

Keywords: Alternative testing methods/strategies, Environmental nanotoxicology, In vitro toxicology, Nanomaterial release

88. Advances in Developing Adverse Outcome Pathways to Assess Inhalation Toxicity of Multi-Wall Carbon Nanotubes

Sybille van den Brule, Giulia Vietti, Dominique Lison. Louvain Centre for Toxicology and Applied Pharmacology, Belgium.

In the nano world, AOPs are being developed to streamline the lung fibrotic hazard of multi-wall carbon nanotubes (MWCNTs). In this talk, I will discuss an AOP that we developed using the in vitro and in vivo toxicological data available in the literature, emphasizing on pathways/endpoints for which correlation between in vitro and in vivo data exists. I will also discuss how the established AOP is being used to establish targeted quantitative in vitro assays to screen a series of MWCNTs differing in their physico-chemical properties to further assess the predictivity of Key Events (KEs) identified. The presentation will also compare our AOP to the AOP published by Labib et al., which was derived primarily from publicly available toxicogenomics data obtained from lungs exposed to MWCNTs and other nanomaterials (NM). The results of the comparison showed that regardless of the approaches used, the AOP and the KEs identified were similar. Labib et al. also demonstrated that lung responses (perturbations of gene expression and toxicity pathways) to MWCNTs were the same regardless of their different properties, thus suggesting a common AOP for NMs of similar class. Contribution of AOP to experimental and regulatory toxicology of NMs, strategies for the development of AOP, as well as associated issues and limitations will be discussed.

Keywords: Adverse outcomes pathway analysis, Carbon-based nanomaterials, Systems biology/toxicology

89. Environmental Applications of Graphene Oxide Architectured Laminates (GOAL)

Chad D. Vecitis. Harvard University, USA.

Here, we present a method to control the interlayer spacing and chemistry of ultrathin graphene oxide architectured laminates (GOAL) on the nm-scale and discuss their potential for advanced water treatment. The simple production method involves bath sonication to mediate graphene oxide (GO) flake dimensions, vacuum filtration of the GO onto a polymer or ceramic membrane followed by UV, HI, or ultrasound reduction to control GO surface chemistry. The GOAL thickness is typically less than 50 nm. The extent of reduction mediates the GOAL interlayer spacing and thus nanopore dimensions on the Angstrom-scale due to elimination of interlayer surface oxy-groups. The nanopore size determines the permeability and selectivity, which makes it a versatile membrane material for advanced water treatment.

Keywords: Carbon-based nanomaterials, Environmental nanotoxicology

90. Prenatal Exposure to Nanomaterials and Effects on Germline and Somatic DNA in the Offspring

Ulla Vogel[1], Karin S Hougaard[1], Håkan Wallin[1], Carole Yauk[2]. [1]National Research Centre for the Working Environment, Denmark; [2]Environmental Health Science and Research Bureau, Health Canada, Canada.

Mounting evidence indicates that disease-associated genetic changes arise during embryonic development, resulting in a varied distribution of genomes throughout the individual and affect both somatic and germ cells. Pulmonary exposure to nanomaterials have been shown to induce inflammation and DNA damage in lung tissue and DNA damage in liver of exposed mice. We therefore wanted to assess whether pulmonary exposure of pregnant mice to diesel exhaust particles (DEP) or the high volume nanomaterials carbon black (CB) and TiO_2 nanoparticles induced DNA damage and mutations in somatic and germline DNA in the exposed offspring. Time-mated pregnant dams were exposed to DEP, CB or TiO_2 NPs by inhalation or repeated instillations. CB and TiO_2 doses corresponded to half of the Danish occuational exposure limit to TiO_2. Increased DNA strand break levels in the comet assay were observed in F1 liver after prenatal exposure to CB but not TiO_2. Germline mutations of the F1 generation was assessed as changes in repeat numbers of expanded simple tandem repeat (ESTR) loci in the F2 relative to their parents. There was no increased tandem repeat mutation in the female germline following prenatal exposure to DEP, CB or TiO_2. Increased mutation was observed in the male germline following exposure to DEP. CB and TiO_2 were not assessed. Daily sperm production was reduced in F2 following CB exposure. In conclusion, we observed DNA damage in somatic DNA and increased mutations in the male germline following prenatal exposure to nanoparticles, but no effects on the female germline.

Keywords: Carcinogenicity, Developmental nanotoxicology, In vivo toxicology

91. Global Gene Expression Profiling in Nanosafety - for Hazard Identification, Grouping and Ranking and Risk Assessment

Ulla Vogel[1], Sarah Søs Poulsen[1], Anne T Saber[1], Nicklas R Jacobsen[1], Carole Yauk[2], Håkan Wallin[1], Sabina Halappanavar[2]. [1]National Research Centre for the Working Environment, Denmark; [2]Environmental Health Science and Research Bureau, Health Canada, Canada.

Risk assessment of nanomaterials faces muliple challenges. One of the first challenges is to identify relevant adverse outcomes. The next challenge is to group and rank different nanomaterials in relation to the specific adverse outcome. We have succesfully employed global transcriptional profiling of target tissues from mice following pulmonary exposure to nanomaterials as a means for both hazard identification, grouping and ranking and for establishing bench mark doses for adverse outcomes in relation to pulmonary exposure to nanomaterials. Mice were exposed at 3 doses and 3 time points to nanomaterials by intratracheal instillation. So far, we have published global transcriptional profiles from lung tissue for 1 carbon black, 5 TiO_2 NPs, 3 MWCNTs, and 4 different sanding dusts from paint with or without TiO_2 NPs. Using global transcriptional profiling, we have shown that pulmonary exposures to nanomaterials induce a pulmonary acute phase response, thus establishing a direct causal link between inhalation of nanomaterials and risk of cardiovascular disease. We furthermore showed that the pulmonary acute phase response was dose-dependent and proportional to the total surface area of pulmonary deposited particles, thus providing a tool for risk assessment of nanomaterials in relation to cardivascular disease. Comparison of transcriptional profiles from different nanomaterials allows grouping of materials according to differentially regulated biological pathways. Using this approach we have shown that 5 different TiO_2 NPs with different size and surface modifications have very similar transcriptional profiles and that two MWCNT with very different physicochemical properties have similar trascriptional profiles.

Keywords: Adverse outcomes pathway analysis, In vivo toxicology, Systems biology/toxicology

92. Toxicity and In Vivo Distribution of Ingested Food-Relevant Inorganic Nanoparticles

James Waldman, Christie McCracken, Andrew Zane, Deborah Knight, Prabir Dutta. Ohio State University Medical Center, USA.

Nanoparticles (NP) are increasingly incorporated into foods, however their biological interactions and potential health impact is poorly understood. We have addressed this issue by assessing the toxicity of food-relevant inorganic NP and mapping their distribution following ingestion. Intestinal epithelial cells exposed for 24 hours to nano-scale TiO_2, SiO_2, ZnO or silver (Ag) exhibited little or no acute toxicity as indicated by assays of necrosis, apoptosis, and metabolic activity even when cells were repeatedly exposed weekly to NP. In contrast, continuous exposure of cells to low concentrations of Ag NP completely inhibited cell proliferation. To map the distribution of ingested NP, SiO_2 NP were synthesized with fluorescent cores and particle surfaces identical to food-grade silica. Following administration of NP to mice by gavage and subsequent euthanasia, laser scanning confocal fluorescence microscopy of tissue sections demonstrated that NP had traversed the intestinal epithelium, passed through the liver to reach the systemic circulation, and accumulated in many organs. Hence, while TiO_2, SiO_2, ZnO or Ag NP are not acutely toxic to intestinal epithelial cells, Ag NP even at very low concentrations completely inhibited epithelial cell proliferation implying potentially serious consequences for stem cells in the intestinal crypts which continuously proliferate to regenerate villous epithelium. In addition, our studies demonstrate that ingested silica NP traverse the intestinal epithelium to enter the systemic circulation and distribute to multiple organs. Whether ingested inorganic NP progressively accumulate in organs and induce inflammatory responses or tissue injury remains to be determined. Funding provided by National Institute of Food and Agriculture.

Keywords: Biodistribution, Metal/metal oxide nanomaterials, ingestion exposure, food-grade nanomaterials

93. A Nanoengineers's Perspective on EHS Topics in Emerging Advanced Nanoengineered Hierarchical Materials

Brian L. Wardle. Massachusetts Institute of Technology, USA.

Bulk nanostructured materials offer tremendous opportunity for re-inventing materials, but also pose many challenges both in terms of characterization, design, processing, scaling and responsible use. Many of these emerging materials and architectures have as yet un-studied exposure and toxicity vectors. This presentation will focus on carbon nanostructure based materials that are emerging as next-generation films and hierarchical architectures (such as nanoengineered advanced composites). We synthesize and experiment on such materials for over a decade and have contributed to exposure understanding for nanomaterials during the course of their development. Both observed mechanical property improvements as well as multifunctional capabilities of nanoengineered composites are pulling such materials forward into practice and into commercial use. I will overview some of the topics we have taken on with the support of industry, including exposures during typical manufacturing and processing, some of the issues we have encountered during the process of increasing our understanding working with the EHS community, and finally give perspective on these and other emergent nanomaterials such as graphene. New research directions, including new 3D nano-scale visualization and contributions in related disciplines such energy storage and transport, will be highlighted if time allows.

Keywords: Emerging nanomaterials

94. Nanoparticle Transport Across the Placental Barrier: Pushing the Field Forward!

Peter Wick, Carina Muoth, Leonie Aengenheister, Melanie Kucki, Tina Buerki-Thurnherr. Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland.

The placenta is a multifunctional organ constituting the barrier between maternal and fetal tissues. Nanoparticles can cross the placental barrier, and there is increasing evidence that the extent of transfer is dependent on particle characteristics and functionalization. Translocated particles may pose risks to the growing fetus but if safe, they might enable new particle-based therapies in pregnancy. In both cases, a comprehensive understanding of nanoparticle uptake, accumulation and translocation is indispensable and requires predictive placental transfer models. The dual perfused ex vivo human placenta system is one of the most advanced human models to study NP translocation. However the availability and throughput capacity limits its broad use. Therefore advanced in vitro models based on human (primary) cells could overcome this limitation. We conduced own studies and evaluate the current literature to draw first conclusions on the possibility to steer translocation of nanoparticles. In addition, it will be discussed if current placental models are suitable for nanoparticle transfer studies and suggest strategies to improve their predictability. This research is supported by funding from the BMBF-project NanoUmwelt (03X0150), the 7th Framework Program of the European Commission (FP7-NANOSOLUTIONS, grant agreement n°309329 and Graphene Flagship, grant agreement n°604391) and Swiss National Science Foundation (NRP-64 program, grant no. 4064-131232).

Keywords: Developmental nanotoxicology, Human toxicology, Toxicological mechanisms

95. Aspect Ratio Plays a Role in the Hazard Potential of CeO_2 Nanoparticles in Cells, Mouse Lung and Zebrafish Gastrointestinal Tract

Tian Xia, Zhaoxia Ji, Xiang Wang. UCLA, USA.

We synthesized a series of cerium oxide (CeO_2) nanorods and nanowires with precisely controlled lengths and aspect ratios. This allows the systematic study of the effect of aspect ratio on in vitro human THP-1 cells and in vivo effects in mouse lung and gastrointestinal tract (GIT) of zebrafish larvae. The cellular study demonstrated that, at lengths ≥200 nm and aspect ratios ≥22, CeO_2 nanorods induced lysosomal damage, pro-inflammatory effects and cytotoxicity. The relatively low "critical" length and aspect ratio were associated with small nanorod diameters (6–10 nm), which facilitates the formation of stacking bundles due to strong van der Waals and dipole–dipole attractions. For in vivo studies, although oropharyngeal aspiration could induce acute lung inflammation for CeO_2 nanospheres and nanorods, only the nanorods with the highest AR induced significant IL-1β and TGF-β1 production in the bronchoalveolar lavage fluid at 21 days, and more collagen production at 44 days was seen with CeO_2 nanorods vs. nanospheres after correcting for Ce lung burden. Using an oral-exposure model in zebrafish larvae, we demonstrated that only highest AR nanorods induced significant growth inhibition, lower body weight, and delayed vertebral calcification compared to CeO_2 nanospheres and shorter nanorods. The key injury mechanism of nanorod was in the epithelial lining of the GIT, which demonstrated blunted microvilli and compromised digestive function. All considered, these data demonstrate that high AR CeO_2 nanorods exhibit more toxicity in the lung and GIT, which could be relevant to inhalation and environmental hazard potential.

Keywords: Adverse outcomes pathway analysis, Alternative testing methods/strategies, Cerium oxide nanoparticles, Toxicological mechanisms

96. Lipid Nanoparticles in Food and Gastrointestinal Tract: Implication in Food Function and Safety

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Nanoemulsions, which consist of nano-sized lipid droplets dispersed within an aqueous medium, are one of the most commonly used types of engineered nanoparticles in food. After ingestion, nanoemulsions are broken down in the gastrointestinal (GI) tract and reassembled into a different type of lipid nanoparticles, mixed micelles. After transport into the enterocytes, the components of mixed micelles are repackaged into yet another type of lipid nanoparticles, chylomicrons. The characteristics of these lipid nanoparticles in food and GI tract have profound effects on the GI fate of many food components. This talk provides an overview on the impact of aforementioned lipid nanoparticles on the absorption, distribution, biotransformation, and potential toxicity of food components. This information is critical for the rationale design of food lipid nanoparticles for enhanced nutrition and food safety.

Keywords: Biodistribution, Nanoemulsion, lipid nanoparticles, micelle, chylomicron

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