3RD NANOSAFETY FORUM FOR YOUNG SCIENTISTS

Valletta, Malta
10-11 September, 2018

Programme Monday 10th September 2018

08:45-09:30	**Registration**	
09:30-09:45	**Welcome**	**Iseult Lynch**
09:45-10:15	**Keynote**	**Mark Miller**
Theme: Understanding the toxicity mechanisms associated with nanomaterial hazard (Chair: Emily Guggenheim)		
10:15-10:30	Targeted Metabolomics: a promising tool to support nanomaterial grouping	Anne Bannuscher
10:30-10:45	Exploring the effect of the medium conditions on the interaction mechanisms between silver nanoparticles and Artificial Model Membranes	Marcos Arribas Perez
10:45-11:00	Evidence for altered genotoxic behaviour of dextran-coated-superparamagnetic-Fe_3O_4 in a Physioxic culturing conditions	Michael K.T. Theodoulides
11:00-11:30	**Coffee Break**	
Theme: Human health and nanomaterials (Chair: Emily Guggenheim)		
11:30-11:45	Comparison of two 3D human lung co-culture models cultured at air-liquid interface to assess the (pro-)fibrotic potential of carbon nanotubes	Hana Barosova
11:45-12:00	Cytotoxicity of different types of layered silicates nanomaterials	Krystyna Maciaszek
12:00-12:15	Interactions of allergens with nanomaterials - structural aspects and biologicals effects	Robert Mills-Goodlet
12:15-12:30	Human dendritic cells (DCs) as target of gold NPs (AuNPs): potential impacts on LPS-induced immune response	Sara Michelini
12:30-14:00	**Lunch**	
Theme: Physicochemical, structural and computational characterisation of nanomaterials (including safer-by-design) (Chair: Sophie M Briffa)		
14:00-14:15	Computational studies of nanoparticle toxicity pathways	Matt Schneemilch
14:15-14:30	Characterization of nanomaterials surface hydrophobicity for risk assessment	Loïc Burr
14:30-14:45	Chemical Characterisation of (Core-Shell) Nanoparticles using PCA assisted ToF-SIMS and XPS	Thomas Heinrich

Programme Monday 10th September 2018

14:45-15:00	Developing spICP-TOF-MS for the Characterization of Multi-element Nanoparticles and Application to Complex Systems	Manuel Montaño
15:00-15:15	Characterization of Nanoparticles and related Metals in Tattoo Ink using Asymmetrical Flow Field-Flow Fractionation coupled with ICP-MS	Roland Drexel
15:15-15:30	Development of protein corona isolation techniques for characterisation with capillary electrophoresis mass spectrometry	Andrew Chetwynd
15:30-16:00	**Coffee Break**	
Theme: Alternative biological systems for nanomaterial hazard assessment (both in vitro and in silico) (Chair: Andrew Chetwynd)		
16:00-16:15	Assessing iron oxide nanoparticle genotoxicity and metabolic changes within an in vitro liver 3D model	Jefferson de Oliveira Mallia
16:15-16:30	Investigating Alternative Models to Evaluate the Impact of Nanomaterials on Neutrophils during Inflammation	Suzanne Gillies
Theme: Risk assessment and standardisation of nanomaterials (Chair: Andrew Chetwynd)		
16:30-16:45	Assessing current regulatory methods for nanomaterial toxicity testing with Daphnia magna: updating traditional methods for novel materials to accurately determine risk	Fatima Nasser
16:45-17:00	In vitro Cytotoxicity of a Water-Stable Covalent Organic Framework	Marisa Sarria Pereira Passos
17:00	**Poster Session**	
19:30	**Conference Dinner – Pepe Nero (Valletta Waterfront)**	

Programme Tuesday 11th September 2018

08:30-09:00	**Registration**	
Theme: Relationship of nanomaterials' physicochemical properties and toxicity (Chair: Nicola William)		
09:00-09:15	Investigations of the neurotoxic effects of engineered nanoparticles in the mouse brain – The N3RvousSystem project	Adriana Sofranko
09:15-09:30	Bio-membrane interaction of silver nanoparticles studied via cyclic voltammetry: effect of nanoparticle coating	Faith Bamiduro
09:30-09:45	Rapid Cyclic Voltammetry: Novel Characterisation of Nanomaterial-Induced Membrane Conformational Dynamics	Sophia Winter
09:45-10:00	The interaction of SiO_2 nanoparticles with the neuronal plasmamembrane: modulation of ionic currents and calcium influx	Marianna Dionisi
10:00-10:15	Exposure medium and nanomaterial aging effect the chronic toxicity of Daphnia magna; a Multigenerational study	Laura-Jayne Ellis
Theme: Data modelling, handling and management (Chair: Tassos Papadiamantis)		
10:15-10:30	Read-across nanoinformatics models for the assessment of NPs zeta potential based on image nanodescriptors	Dimitra-Danai Varsou
10:30-10:45	Adaptation of geochemical modelling tools to predict bioavailable metal concentrations in agricultural soils amended with metal oxide nanoparticles	Sónia Morais Rodrigues
10:45-11:00	Subspace Clustering as a tool for the Read-Across and Categorization of Nanomaterials	Gianpietro Basei
11:00-11:30	**Coffee Break**	
Theme: Nanomaterials in the environment (Chair: Marta Baccaro)		
11:30-11:45	Modelling nanoparticle transport and bio-availability in soil mesocosms	Geert Cornelis
11:45-12:00	Multigenerational exposure of Folsomia candida to copper agrochemicals: conventional and nano-pesticides	Joana Neves

Programme Tuesday 11th September 2018

12:00-12:15	3D Chemical imaging with ToF-SIMS to elucidate TiO_2 NPs and freshwater algae interactions	Pietro Benettoni
12:15-12:30	Laser desorption ionization mass spectrometry as a useful tool for nanoparticle coating characterization	Konstantinos Giannopoulos
12:30-14:00	**Lunch**	
14:00-14:30	**Keynote**	**Susana Loueiro**
14:30-15:00	**Closing Remarks & Awards**	
15:00-15:30	**Coffee Break**	
15:30-17:30	**DLS theory session**	**Malvern Pananalytic**

Posters

Theme: Understanding the toxicity mechanisms associated with nanomaterial hazard.		
1.	Life span-resolved nanotoxicology in the nematode C. elegans: the gut – neural axis	Annette Piechulek
2.	Toxicokinetics of silver nanoparticle effects to the nematode C. elegans	Carolin Schultz
3.	Coating matters: An electrochemical based study of effect of coating in CeO2 NPs with model membranes.	Natalia Domenech-Garcia
4.	A Model for Competitive Adsorption in Blood Plasma and Lung Lining Fluid	Stefano Poggio
5.	Mechanistic insights into aluminum nanomaterial uptake and metabolism after co-exposure to vitamin A and D	Yves Hachenberger
Theme: Human Health and nanomaterials		
6.	Advanced in vitro cell culture module for long-term cultivation and toxicity screening of nanomaterials	Michelle Hesler
7.	Human renal proximal tubule epithelial TH1 cells as in vitro kidney model	Patricia Bégerová
8.	The challenge of detecting engineered nanomaterials in biological matrices – From sample preparation to characterization via Field-Flow Fractionation	Roland Drexel
9.	The intrinsic metal content of individual A549 cells as a baseline for cellular exposure to metal nanoparticles	Benjamin Fryer
Theme: Physicochemical, structural and computational characterisation of nanomaterials (including safer-by-design)		
10.	Optimizing concentration of biological media at different temperatures for silica nanoparticle stability	Pirutchada Musigapong
Theme: Screening nanotechnology for nanomaterials		
11.	Development of a microfluidic flow system for toxicity screening of nanomaterials	Joshua Owen
12.	Enhancing the use of *in vitro* (neutrophil) and zebrafish embryo models as alternatives to rodent testing for assessing immunological responses to nanomaterials (NMs)	Suzanne Gillies
Theme: Standardisation of analytical methodology and protocols		
13.	Are existing standard methods suitable for the evaluation of nanomedicines: some case studies	Sabrina Gioria
14.	The development of a guidance protocol for selection of the most appropriate and effective methods for detecting reactive oxygen species and oxidative stress in response to nanomaterials	Veronica Turcu

Posters

Theme: Data modelling, handling and management		
15.	ACEnano Knowledge Warehouse to support documentation and collection of nanomaterials physicochemical characterisation data	Lucian Farcal
16.	Read-across in silico investigation of the bioactivity and toxicity behaviour of carbon nanotubes	Dimitra-Danai Varsou
17.	Making use of available and future data to predict the properties, interactions and hazards of engineered nanomaterials by means of in silico tools: a critical review	Gianpietro Basei
Theme: Nanomaterials Exposure and Fate		
18.	Cytotoxicity of Silver Nanoparticles: Zebrafish cells a new experimental model to evaluate nanoparticles' toxicity	Ana Isabel Carrazco Quevedo
19.	Sulfidized silver nanoparticles induce lower toxicity than pristine ones to the pond snail Physa acuta	Carlos Pinheiro
20.	The influence of differing soil properties on the uptake of different Ag nanoparticle forms from soil by plants exposed from seed	Elma Lahive
21.	Comparison of the toxicity and bioaccumulation of different types of Cd-based Quantum Dots for model plants	Pavlína Modlitbová
22.	Determination of spatial distribution of selected lanthanides contained in upconverting nanoparticles in plant tissues by laser induced breakdown spectroscopy	Tereza Švestková
23.	Shape Dependent Transformation and Translocation of Ceria Nanoparticles in Plant	Peng Zhang
24.	Microscopy methods for assessing the biological uptake and effects of ENP	Emily Guggenheim
Theme: Nanomaterials in the environment		
25.	New insights into the interaction of GMs with bacteria film	Zhiling Guo
26.	Effect of electric current and zero-valent iron on bacterial consortia in site polluted by chlorinated ethenes	Nhung H.A. Nguyen
27.	A comparison of analytical techniques for measuring the attachment rate of nanomaterials to soil in kinetic batch tests	Jessica L. Adams

Keynotes

Dr Mark R. Miller – *"Nanoparticles and the cardiovascular system: a particularly small problem"*

Mark Miller is a Senior Research Scientist working in the Centre for Cardiovascular Science at the University of Edinburgh, United Kingdom.

Mark's research investigates the effects of air pollution on the cardiovascular system (the heart, blood vessels and blood). His main focus is the biological pathways by which the particles in vehicle exhaust can cause heart disease. He also has an interest in the potential for manufactured nanoparticles to cause harm to the cardiovascular system.

Mark's research findings have received extensive coverage in the national and international media. His work contributed to the Department of Cardiology's Queens Anniversary Award 2014-16 for outstanding contribution to scientific research. He is an Editor of the journals Particle & Fibre Toxicology and Frontiers in Cardiovascular Medicine, as well as an Expert Member of COMEAP, the UK governmental advisory Committee on the Medical Effects of Air Pollution. His work is predominantly funded by grants from the British Heart Foundation.

Ass Prof Susana Loureiro – *"Nanomaterials hazard assessment: understanding the nano to predict the effects"*

Susana Loureiro is an Assistant Professor with Habilitation at the Biology Department, University of Aveiro and the coordinator of the Thematic Line Ecology and Functional Biodiversity of CESAM-Centre for Environmental and Marine Studies.

Susana Loureiro started her undergraduate studies at the University of Coimbra, in Biology, following an MSc in Ecology at the same university. In 2000 she started a PhD in Biology at the University of Aveiro. Her PhD focused on the hazard assessment of soils from a mine in the northeast of Portugal, Jales Mine. In 2005, she was hired as Assistant Researcher at the University of Aveiro, at CESAM & Department of Biology.

Her main topics of research are related to mixture toxicity assessment, combined effects of chemicals and natural stressors, multigenerational effects, long-term effects, and nano-ecotoxicology, including both fate and effects of nanomaterials.

Oral Presentations

Targeted Metabolomics: a promising tool to support nanomaterial grouping

Anne Bannuscher[1], Aileen Bahl, Katja Kettler[1], Andreas Luch[1] and Andrea Haase[1]

1 German Federal Institute for Risk Assessment (BfR), Department of Chemical and Product Safety, Berlin, Germany

Nanomaterials (NM) can be produced in many different variants and are widely used. But toxicity testing of each variant is not feasible. So, grouping approaches for NMs are urgently needed. Metabolomics analyses are particularly useful because they provide insights into modes of action (MoAs) and support the categorization of NM. Within the project NanoToxClass we used a targeted metabolomics approach to screen 12 different NM in two cell lines from the alveolar region of the rat, on the one hand epithelial cells and on the other hand macrophages. The study included seven systematically selected SiO_2 variants differing in size, charge and surface coating and five other NMs, i.e. two organic pigments, TiO_2 NM-105, Graphene oxide and Mn_2O_3. Our results show that NMs can be categorized due to changes in the metabolic profile. These responses varied, as expected, within the different NMs and the two cell types. However, we also noticed similarities of induced responses such as upregulation of several phospholipids or downregulation of biogenic amines in both cell lines by e.g. SiO_2_7. From the results, we selected NMs for detailed investigations of dose- and time-responses in vitro and for in vivo studies. Overall, our results enabled important insights into the underlying MoAs and allowed for first categorization based on changes of metabolic profiles.

Exploring the effect of the medium conditions on the interaction mechanisms between silver nanoparticles and Artificial Model Membranes

Marcos Arribas Perez[1], Paul Beales[1] and Andrew Nelson[1]

1 School of Chemistry University of Leeds, Leeds, United Kingdom

The wide variety of nanoparticle (NPs) designs, their sensitivity to environmental conditions and the complexity of biomembranes lead to a high variability of NP-biomembrane interaction mechanisms which may induce cytotoxicity. Therefore, the development of toxicity screening methods is essential to reduce health and environmental risks derived from the use of NPs. The present work is a biophysical approach to the interaction between silver nanoparticles (AgNPs) and artificial model membranes in different media. This investigation will assist to develop the multimodular High Level Integrated Sensor for Nanotoxicity Screening (HISENTS) by helping to interpret the rapid cyclic voltammetry (RCV) outcomes from the electrochemical lipid-on-mercury module. The effect of AgNPs on Large Unilamellar Vesicles (LUVs) was investigated by fluorescence spectroscopy techniques. Those experiments were complemented with confocal microscopy observations using Giant Unilamellar Vesicles (GUVs). Additionally, these observations were correlated with the RCV outcomes from the HISENTS electrochemical module. The composition of the media influences the AgNPs-membrane interaction. In high ionic strength buffer, AgNPs induced an increase in membrane permeability whereas in glucose solution the membrane barrier function was not affected. These observations indicate that sugars could be either affecting the physicochemical properties of AgNPs or acting as a protective shield around membranes.

Evidence for altered genotoxic behaviour of dextran-coated-superparamagnetic-Fe_3O_4 in a Physioxic culturing conditions

Michael K.T. Theodoulides[1,2], Martin.J.D.Clift[1], Rachel Adams[2], Sandor Balog[3], Richard Webb[2] and Shareen H. Doak[1]

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2 Department of Biomedical Sciences, Cardiff Metropolitan University, UK

3 Adolphe Merkle Institute, University of Fribourg, Switzerland

Iron oxide nanoparticles are intended for use in several medical applications, leading to inevitable human exposure. The aim of this study was to evaluate the range of toxic responses of dextran-coated-superparamagnetic-Fe_3O_4 nanoparticles (dSPIONs) on monocytes (THP-1), macrophages (dTHP-1) and hepatocarcinoma (HepG2) cells in physioxia ($5\%O_2/5\%CO_2$) and hyperoxia ($21\%O_2/5\%CO_2$). The relationship between cellular calcium homeostasis ([Ca2+]cyto) and potential genotoxicity was explored. dSPIONs hydrodynamic diameter (88.6±8.3nm) and ζ-potential (+10.4±1.3mV) were determined by dynamic light scattering. Significant concentration and cell-type-dependent (dTHP>THP-1>HepG2) increases in dSPION-cellular interaction following 24h exposure (0-100μg/ml) were measured using the Ferrozine assay. Generally, dSPION cellular interaction were significantly ($p<0.05$) greater in physioxia compared to hyperoxia. dSPION-cellular interaction correlated with chromosomal damage in HepG2 cells, determined by the cytokinesis-block-micronucleus-assay. Significant increases in genotoxicity, coupled to a reduction in cell viability (relative-population-doubling) were only observed under physioxic conditions. Significant increase in tumour-necrosis-factor-α and interleukin-8 secretion from all cell lines and conditions correlated with observed HepG2 genotoxicity. In all cell-types/environments, dSPIONs induced cell-dependent increases in $[Ca^{2+}]$cyto for up to 5h, a disrupted homeostasis was not identified after 24h exposure. In addition to dSPIONs cell-type specific biological interaction/impact, results indicate that environmental oxygen concentration has a significant role in the NP-cell interaction/impact.

Comparison of two 3D human lung co-culture models cultured at air-liquid interface to assess the (pro-)fibrotic potential of carbon nanotubes

Hana Barosova[1], Anna Maione[2], Dedy Septiadi[1], Monita Sharma[3], Amy J. Clippinger[3], Alke Petri-Fink[1], Patrick Hayden[2] and Barbara Rothen-Rutishauser[1]

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Introduction: The use of multi-walled carbon nanotubes (MWCNTs) in commercial products is increasing, thus human exposure can occur during production or life-cycle primarily via inhalation causing potential adverse effects such as pulmonary fibrosis. Therefore, there is a need to design human-relevant in vitro testing and exposure strategies to assess their effects.

Materials and Methods: Two human-based co-culture models, one with cell-lines (epithelial cells (A549), fibroblasts (MRC-5), and macrophages (THP-1)) and one with primary cells (MatTek Corporation; primary human lung endothelial and epithelial cells, fibroblasts, and macrophages) were exposed to aerosolized MWCNTs at air-liquid interface using the VITROCELL© Cloud system enabling repeated deposition of low concentrations.

Results: The cell-line co-culture model showed upregulation of (pro-)inflammatory but not (pro-)fibrotic cytokines upon exposures to MWCNTs for up to 96h. Repeated exposures of the primary cell-based model to transforming growth factor-β (fibrotic positive control) for up to 21 days showed an increase in pro-fibrotic markers (Collagen, Type I and fibronectin).

Conclusion: The primary cell co-culture model is more suitable for predicting the development of pulmonary fibrosis. In combination with other in vitro and in silico methods, the co-culture models can aid in the hazard assessment of e.g. nanomaterials.

Cytotoxicity of different types of layered silicates nanomaterials

Krystyna Maciaszek, David Brown and Vicki Stone

Heriot-Watt University, Edinburgh, UK

Background: The highly attractive properties of layered silicates has resulted in continuous expansion of these materials in a wide range of applications. However, their potential toxicological effects on human health are not fully investigated. Concerns, regarding layered silicates, are associated with their: (i) nanoscale size, (ii) high aspect ratio resulted from their platelet geometry (thickness of about 1 nm and length and/or width of up to several microns) and (iii) chemical composition.

Methods: A wide range of synthetic layered silicates (SLS) and natural layered silicates (NLS) was investigated through in vitro toxicological studies using mouse monocyte macrophage cell line (J774A.1) and human primary macrophages, following 24 h exposure. Different end points such as cytotoxicity, inflammatory responses and impact of SLS and NLS particles on cell morphology were examined. The panel of particles tested consists of SLS and NLS varying in different physicochemical properties such as size, charge, chemical composition.

Results: All types of NLS and SLS induce dose-dependent cytotoxic effect and inflammatory responses, but the degree of induction was varying depending on the type of layered silicate. Moreover, the light microscopic, SEM and TEM examination revealed that treatment of macrophages with layered silicates caused the formation of vacuoles. Vacuole formation and vacuoles size distribution were both time and dose dependent.

Conclusions: These initial results show that SLS and NLS vary in their potential cytotoxicity to macrophages in vitro, according to the physicochemical characteristics of the particles.

Interactions of allergens with nanomaterials - structural aspects and biologicals effects

Robert Mills-Goodlet[1], Milena Schenck[2], Mark Geppert[1], Martin Himly[1] and Albert Duschl[2].

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2 University of Salzburg Department Chemistry and Physics of Materials, Salzburg, Austria

Allergens are highly abundant and nanoparticles (NPs) are becoming more omnipresent every day, in particular airborne particles. This increases the likelihood of being exposed to them either at the workplace environment or in everyday life. Contact between allergens and NPs becomes more likely resulting in a non-covalent allergen coating of NP known as bio-corona formation. This can induce structural alterations of coated allergens and may impact their biological effects. This study investigates the interactions of allergen-NP conjugates focusing on binding affinity, conformational changes, and uptake into epithelial cells. The binding properties of recombinant allergens and allergen extracts to porous SiO2 NPs were determined using SDS-PAGE and CD spectroscopy. An apparent selectivity for the major birch pollen allergen Bet v 1 was observed concomitant with structural alterations upon binding. The impact of allergen-NP conjugates on uptake by the recently established human type I Alveolar Epithelial Lentiviral-immortalized (hAELVi) cell line was determined using flow cytometry and confocal laser scanning microscopy. A modulation of the uptake kinetics of fluorescently labelled Bet v 1 was observed when bound to SiO_2 NPs: unbound allergens was taken up slowly and continuously for 24 h, whereas allergen-NP conjugates were taken up rapidly reaching saturation within 2 h. Moreover, NP-conjugated allergens induced an upregulation of pro-inflammatory cytokines compared to unbound allergen, as evidenced by qPCR. In conclusion, modulations in protein delivery and pro-inflammatory responses at the lung epithelial barrier should be taken into consideration for nanosafety assessment.

Human dendritic cells (DCs) as target of gold NPs (AuNPs): potential impacts on LPS-induced immune response

Sara Michelini[1], Alessandra Prinelli[2], Muamera Sarajlic[1], Albert Duschl[1] and Jutta Horejs-Höck[1].

1 Paris Lodron Universität Salzburg, Salzburg, Austria

2 AvantiCell Science Ltd, Ayr, UK.

Introduction: DCs are professional APCs, there is, therefore, an urgent need to clarify AuNPs impacts on those cells, especially in non-homeostatic conditions. This study aims to assess the impact of AuNPs of different sizes, on the maturation of LPS-induced DCs with the final purpose of identifying markers for NPs risk assessment.

Materials and methods: Monocyte-derived DCs (moDC) were cultivated for 48h either in presence of LPS or AuNPs of different sizes or with the combination of the two. Subsequently, surface markers expression and cytokine production were assessed via flow cytometry and multiplex immunoassay respectively.

Results: Markers that allowed to follow DC differentiation in presence of nanoparticles included CD83, CD86, ILT3, ILT4, HLA-DR and PD-L2, plus the secreted cytokine IL-12. Treatment of human moDCs with LPS in combination with 26nm-AuNPs induced a tolerogenic-like phenotype characterized especially by a lower production of CD86 and IL-12 with concomitant upregulation of ILT3. We will follow up indications that this effect is size-specific.

Conclusion: Taken together these data support the hypothesis that AuNPs of defined sizes can have specific properties, perhaps in terms of surface area, which boost their anti-inflammatory activity.

Computational studies of nanoparticle toxicity pathways

Matt Schneemilch

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Our research employs three approaches to gauge nanoparticle toxicity. Atomistic Molecular Dynamics simulations of lipid bilayer adhesion on solid substrates, using a novel simulation geometry, directly measure, for the first time, the strength of adhesion on a range of typical nanomaterials. The force fields are carefully optimised to reproduce relevant interfacial properties such as the heat of immersion and interfacial water density profiles and the adhesion strength is directly linked to microscopic surface structure. The results feed into our second approach, where coarse-grained molecular dynamics simulations are used to study, for the first time, full wrapping of particles by bilayers. This allows us to interrogate theoretical phase diagrams of wrapping behaviour which apply to infinitesimal membranes but break down when the particle size is comparable to the bilayer thickness, and to investigate the role of particle morphology. Both approaches are particularly important in toxicity pathways involving direct contact between particle surfaces and cell membranes such as lysosome rupture in macrophage overload. Finally, we investigate the formation of Reactive Oxygen Species through calculations of the electronic structure of small nanoparticles using Density Functional Theory, thought to occur when the absolute conduction band minimum potential overlaps with the cellular redox potential.

Characterization of nanomaterials surface hydrophobicity for risk assessment

Loïc Burr[1], David Schmid[1], Stephano Cattaneo[1] and Silvia Generelli[1]

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With the increasing usage of nanoparticles, nanomaterials risk assessment is crucial to create new adequate regulations, yet missing due to the absence of reproducible and standardized detection and characterization techniques. Surface properties of nanomaterials are key determinants of their transport, interaction and toxicity. In particular, nanomaterials surface hydrophobicity influences their binding to organic and biological molecules such as proteins and subsequently their fate in the human body. In the frame of the European ACEnano project, CSEM evaluates novel approaches and develops methods to assess nanomaterials surface hydrophobicity by dye-loaded Fluid Flow Fractionation (FFF), Hydrophobic Interaction Chromatography (HIC) and Wavelength Interrogated Optical Sensing (WIOS). WIOS uses the evanescent field to probe changes induced by nanoparticle adsorption at the interface of a waveguide layer. First, the results of the evaluation of the dye-loaded FFF technique for hydrophobicity sensing will be detailed. Then, the HIC and WIOS techniques will be presented as well as preliminary results on the characterization of the hydrophobicity of well-defined nanoparticles.

Chemical Characterisation of (Core-Shell) Nanoparticles using PCA assisted ToF-SIMS and XPS

Thomas Heinrich[1], Anja Müller[1], Markus Schneider[1], Katja Sparnacci[2] and Wolfgang Unger[1]

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The analysis of nanomaterials is currently an important task - especially in case of risk assessment – as the properties of these material class are not well understood. The rather high surface area of these objects renders their interactions significantly different to their corresponding bulk. Thus, the surface's chemical composition must be investigated to get a better understanding and prediction of the nanomaterials' behavior. ToF-SIMS and XPS have proven to be powerful tools to determine the general chemical composition. The superior surface sensitivity of ToF-SIMS furthermore allows us to study mainly the utmost atomic layers and thus gives us an idea of the interactions involved. Here, we present initial data on the analysis of Hyflon®-polystyrene core-shell nanoparticles which can be used as a model system due to the known preparation and a rather good chemical as well as physical separation of core and shell. Furthermore, results on Au nanoparticles with and without an antibody shell are presented. Principle component analysis (PCA) will be used to detect the influence of sample preparation and for a better separation of different samples. ToF-SIMS imaging is desired to be implemented for single particle detection as well.

Developing spICP-TOF-MS for the Characterization of Multi-element Nanoparticles and Application to Complex Systems

Manuel David Montaño[1], Nathalie Tepe[1], Thilo Hofmann[1] and Frank von der Kammer[1].

1 University of Vienna, Vienna, Austria

The entry of engineered nanoparticles (ENPs) into the environment has necessitated the development of sophisticated analytical techniques and methods for detecting and characterizing them in complex matrices and at low concentrations. Environmental matrices contain a large number of naturally occurring nanoparticles (NNPs) with compositions, sizes, and morphologies similar to their engineered analogues, further impeding accurate and precise ENP detection. The development of single particle ICP-time-of-flight-mass spectrometry (spICP-TOF-MS) open the doors for differentiating ENP and NNP populations based of their elemental composition, as the spICP-TOF-MS permits the quasi-simultaneous detection of nearly the entire atomic mass range (7-250 m/z+) on a single particle basis. This can be further combined with pre-fractionation techniques such as field flow fractionation to better resolve these differences. In this study, mixtures of various multi-element containing particles were studied to assess the performance of spICP-TOF-MS in accurately characterizing NP suspensions and explored the feasibility of developing a spectral library that can be used to fingerprint ENP-containing environmental sites.

Characterization of Nanoparticles and related Metals in Tattoo Ink using Asymmetrical Flow Field-Flow Fractionation coupled with ICP-MS

Florian Meier[1], Roland Drexel[1], Evelin Moldenhauer[1], Tony Pfaffe[1], Thorsten Klein[1]

1 Postnova Analytics GmbH, Landsberg, Germany

With increasing applications for nanoparticles in various consumer products, there is an urgent need for accurate and robust characterization methods for these materials also in complex matrices. Field-Flow Fractionation (FFF) is a particle separation and characterization technique that works over a broad size range, usually from 1 nm to 30 µm [1,2]. Coupled with ICP-MS, FFF not only enables the fractionation of sample constituents according to their size, but also provides information about elemental distributions across particle size distributions [3-4]. Metal and metal oxide nanoparticles are added to tattoo inks to enhance color vibrancy, but cutaneous allergies may occur due to the presence of toxic metals in inks. This presentation demonstrates the use of Asymmetrical Flow FFF (AF4) coupled with ICP-MS to characterize several types of tattoo ink by metal nanoparticle composition. Particle size and element distributions of various metals (Al, Ti, Cu) were measured to study the composition of ink ingredients as a function of particle size, and dissolved versus particulate metal components. This is the first application of AF4-ICP-MS to metal nanoparticle analysis in tattoo ink.

Development of protein corona isolation techniques for characterisation with capillary electrophoresis mass spectrometry

Klaus Faserl[1], Andrew J. Chetwynd[2], Iseult Lynch[3], James A. Thorn[2] and Herbert H. Lindner[1].

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Since the nanoparticle corona rose to eminence a decade ago, it has been widely investigated to characterise and derive its significance. However, work to determine the sample preparation workflow which gives the most complete picture of the protein corona has not been reported. The most published approach requires boiling NP-corona complexes in SDS buffer and running a 1D SDS-PAGE where bands of interest are excised and digested prior to LC-MS analysis. This method only characterizes a limited number of high abundant corona proteins. The aim of the current work is to characterise both high and low abundance proteins. It also showcases the first application of capillary electrophoresis (CE) for the analysis of the protein corona. The off-particle digest approach with SDS-PAGE with an in-gel digest was compared with an on-particle digest where the corona proteins were digested in-situ. All samples were analyzed by CESI-MS using a CESI 8000 Plus connected to a Thermo QE HF Orbitrap. Separation was performed with a neutral capillary with 30kV separation voltage and 2 PSI pressure. The optimized on-particle digest gave 2215 peptides, 1000 more than the off-particle approach. This allowed identification of over 200 protein groups, over 50 more than the off-particle method. Furthermore, the on-particle workflow could be completed in under a day, nearly twice as quickly as that of the off-particle.

Assessing iron oxide nanoparticle genotoxicity and metabolic changes within an in vitro liver 3D model

Jefferson de Oliveira Mallia[1], Sylvain Bohic[2], Jean-Luc Ravanat[3], Neenu Singh[4], Ume-Kulsoom Shah[1], Gareth Jenkins[1] and Shareen H. Doak[1].

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In vivo studies have shown that the liver is one of the primary sites of nanoparticle accumulation. In this study, HepG2 liver spheroids were used as an alternative in vitro model to assess iron oxide (magnetite and maghemite) nanoparticle hepatic interactions. Synchrotron X-ray fluorescence (SXRF) assessed nanoparticle distribution and metal homeostasis. Liver function metabolites; albumin, urea and aspartate transaminase (AST) were quantified using colorimetric assays. Cytokinesis block micronucleus assays and single-cell gel electrophoresis (COMET) were used to assess genotoxicity. SXRF studies showed a dose-dependency accumulation of nanoparticles in tissue sections. This accumulation also affected metal homeostasis by increasing intracellular copper levels. Spheroids exposed to 100 µg/ mL (top dose) maghemite showed a 3-fold increase in AST in 24 h and a 2-fold increase for albumin and urea secretion in 48 h, compared to controls. Maghemite induced a significant increase in micronuclei frequency from the lowest dose, reaching 2.1-fold at the top dose. Magnetite only induced significant increases at the top dose (2.2-fold). COMET scores showed no significant increases. In conclusion, the metabolic data shows that the iron oxide nanoparticles are inducing effects similar to iron overload responses, disturbing iron homeostasis, coupled with increased micronucleus frequencies.

Investigating Alternative Models to Evaluate the Impact of Nanomaterials on Neutrophils during Inflammation

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Assessment of nanomaterial (NM) safety has routinely relied on rodent testing via the evaluation of inflammatory responses. Here, we investigate alternative in vitro and in vivo models for assessing the stimulation and resolution of NM-mediated inflammation, with a focus on the neutrophil. We compare inflammatory responses of the human HL-60 neutrophil-like cell line, with those of primary neutrophils isolated from human blood. We tested a panel of NMs (Ag, ZnO, TiO_2, CuO and MWCNTs), and assessed NM-toxicity using several endpoints including cytotoxicity, cytokine production and respiratory burst activation. We found the responses of the cell line to be comparable with primary cells, with the exception of ZnO particles, which interestingly were less toxic to primary neutrophils than to the cell line. We also present preliminary data demonstrating the use of larval zebrafish to study in vivo responses of NM-mediated inflammation. The accumulation of fluorescently labelled neutrophils was tracked in Tg(mpx:GFP) larvae, following microinjection of NMs. Our results suggest that these alternative models exhibit a similar pattern of NM-toxicity as seen in rodent models. Their use should be encouraged to better align nanotoxicity testing with the principles of the 3Rs; to Reduce, Refine and Replace animal testing in research.

Assessing current regulatory methods for nanomaterial toxicity testing with Daphnia magna: updating traditional methods for novel materials to accurately determine risk

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Nanomaterials (NMs) are being widely incorporated into a variety of fields due to their unique qualities provided by their small size and high surface area to volume ratio. The increased use of NMs leads to their inevitable deposition into environmental waters where they interact with environmental organisms such as the fresh water zooplankton Daphnia manga (D. manga). D. magna is an ideal candidate for fresh water toxicity testing and a central study species used by the Organization for Economic Cooperation Development (OECD) which sets the gold standards for eco-toxicity testing protocols. These protocols were originally designed for bulk chemicals though have been deemed acceptable for NMs. These protocols fail to account for key exposure features for NMs, such as access to food to push out previously accumulated matter during the natural clearance processes, and that NMs will have acquired a corona of biomolecules that changes their identity, agglomeration, uptake and excretion. Thus, the absence of these factors can lead to significant over or underestimation of NMs taken up and retained within D. magna. Herein we present evidence to support the call for revised guidelines for D. magna acute and chronic toxicity tests for NMs by looking at two different sized polystyrene (PS) NMs under more realistic environmental conditions.

In vitro Cytotoxicity of a Water-Stable Covalent Organic Framework

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Porous nanostructured materials have increasingly attracted interest in the literature due to their properties [1]. Yet, while efforts have been focused on identifying potential (bio)applications of this emergent class of materials, data related to their biocompatibility and toxicity are scarce and remains poorly understood. Our research group has recently demonstrated the solid-phase adsorption valence of a 2D water-stable covalent organic framework (COF) - TpBD-Me2 [2]. Hence, COF and respective building blocks were characterized for its biocompatibility via in vitro cell-based tests. Cellular metabolic activity was measured as an indicator of cytotoxicity. Human normal epithelia and colon colorectal cancer cells Caco-2 were incubated with different concentrations of COF, ranged from 0.001 to 100 µg.mL-1. Tp building block was tested for concentrations ranged from 0.1 to 20 µg.mL—1 and BD-(OMe)2 from 1 to 80 µg.mL—1. Presto blue cell viability protocol was applied and fluorescence was measured at λex 560; λem 590 nm. Human non-small cell lung adenocarcinoma A549 was further considered as model for COF cytotoxicity profiling. Moreover, macrophages cytotoxic response was screened. MTT cell proliferation assay was considered. Absorbance at λ 570 nm was measured. Overall, COF material did not reveal significant cytotoxicity for any of the models tested.

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Investigations of the neurotoxic effects of engineered nanoparticles in the mouse brain – The N3RvousSystem project

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A specific goal of the research project N3RvousSystem is the development of a strategy for the detection and evaluation of potential adverse effects in the brain following oral exposure to nanoparticles (NPs). Neurotoxicant-induced effects on neurophysiology are often reflected in behavioural impairments that precede neuropathological changes. In the current studies, NP-induced effects on behaviour and neuropathology were explored in C57BL/6J mice following protocols based on the OECD guideline for the testing of neurotoxicity in rodents (#424). These studies involve ad libitum exposures to pellets dosed with either CeO_2-NPs (NM-212, EU-JRC repository), TiO_2-NPs (NM105) or Ag-NPs (Aldrich, #576832) in different concentrations or control pellets. Using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) and scanning electron microscopy (SEM) the NPs were characterized in their pristine form. The feasibility of in situ detection by ToF-SIMS and SEM was demonstrated for CeO_2-NPs in feed pellets. Both methods were also used for in situ detection of these NPs in mouse brain and intestine. In mice exposed to CeO_2-NPs (1 or 10 mg/g feed pellets) no major behavioural impairments could be observed. Moreover, immunohistochemical analyses revealed no alterations in the level of neuroinflammation. Investigations with TiO_2-NPs and Ag-NPs are ongoing and results will be presented.

Bio-membrane interaction of silver nanoparticles studied via cyclic voltammetry: effect of nanoparticle coating

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Nanoparticle manufacturing processes often use additives to engineer / preserve vital properties of the nanoparticles such as size, shape and degree of dispersion. These additives usually function by adsorbing onto the nanoparticle surface. Trisodium citrate, polyvinyl pyrrolidone and tannic acid are commonly used for silver nanoparticle suspensions[1,2]. The potential toxicity of nanoparticles can be studied via in-vivo and/or in-vitro observation of their interaction with a biological entity. While there may be interest in studying nanoparticles without coating, it is often difficult to achieve experimentally, as particles still acquire a 'coat' from the media in which they are suspended. In addition to particle size, concentration, and type of suspension medium, factors such as zeta potential and type of coating could influence the nature and extent of nanoparticle-membrane interactions leading to variant responses by nanomaterials of similar characteristics (size, shape, and concentration). Results of interaction between ~ 30 nm silver nanoparticles with different surface coatings and a phospholipid membrane system observed via rapid cyclic voltammetry will be presented, and resulting implications for interpreting nanoparticle toxicity discussed.

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Rapid Cyclic Voltammetry: Novel Characterisation of Nanomaterial-Induced Membrane Conformational Dynamics

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Background: Real-time measurements from HISENTS' unique high-throughput electrochemical sensor address pivotal impediments of membrane morphology, in conjunction with fluorimetric assessment of vesicle-encapsulated fluorophores, to evaluate nanoparticle perturbation of equilibrium lipid ordering crucial to standard biological function.

Materials and Method: 400 nm DOPC LUVs were prepared and encapsulated with 5(6)-Carboxyfluorescein in HEPES buffer, then introduced to citrate-stabilised gold nanospheres (diameters 5 to 50 nm; concentrations up to 120 µmol dm-3) for a 30-minute incubation period monitored fluorometrically. Non-encapsulated Laurdan-integrated LUVs were also assessed in this manner. These support parallel HISENTS' RCV measurements (DOPC on mercury and gold nanoparticle concentrations ranging from 6 to 120 µmol dm-3), comparing nanospheres and nanorods. TEM images were obtained alongside DLS vesicle studies.

Results: Across all techniques, lipid disordering and structural reformation durations increased with greater nanoparticle concentrations, up to a certain threshold, at which point the lipid reached a state irreversible to its initial order prior to interaction.

Conclusion: The disruption of lipid ordering and self-repairing capacity are potentially significant physiological nanomaterial properties, which in living organisms could induce cell damage. The RCV technology has excellent scope for further analysis, including silver and silica nanoparticles alongside confocal microscopy and epifluorescence.

The interaction of SiO_2 nanoparticles with the neuronal plasmamembrane: modulation of ionic currents and calcium influx

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**Marianna Dionisi and Carla Distasi contributed equally to this work.*

Introduction: SiO_2 nanoparticles (SiO_2 NPs) are one of the most promising tools in the field of nanomedicine. We have previously shown that non-toxic doses of SiO_2 NPs (50 nm) induce membrane potential depolarization, modulating the electrical activity of neuroendocrine cells. Since this process is dependent on Ca^{2+} influx, we investigated which calcium-permeable channels are activated by SiO_2 NPs.

Materials & Methods: Combining Ca^{2+} imaging and patch clamp techniques with a pharmacological approach, we obtained a detailed biophysical characterization of the multiple pathways activated by SiO_2 NPs.

Results: Both Ca^{2+} imaging data on cell populations and electrophysiological recordings at single channel and whole cell levels suggest that TRPV4, Cx and Panx channels are the major components of inward currents elicited by SiO_2 NPs. Furthermore, pre-incubation with the antioxidant N-acetyl-L-cysteine (NAC) strongly reduce $[Ca^{2+}]i$ increase.

Conclusions: Our findings suggest that SiO_2 NPs directly activate a complex set of calcium-permeable channels, possibly by free radicals production. The mechanisms of interaction between the SiO_2 NPs and their targets is a prerequisite to the rational design of safe and efficient nanotools for laboratory and clinical applications.

Exposure medium and nanomaterial aging effect the chronic toxicity of Daphnia magna; a Multigenerational study

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Daphnia magna reproduce parthenogenetically, which is well suited for experimental genetic studies and monitoring stress/adaptive change to their environments. We investigated key biological endpoints, including morphology, survival, growth, reproduction, and gene expression of related molecular pathways in response to exposure pristine and aged silver (AgNPs) and titanium dioxide (TiO_2) nanoparticles (NPs), with a range of surface coatings. Our aims were to identify specific stress responses to understand if: (1) different NP compositions induce the same pathways and effects; (2) if exposure in the presence of natural organic matter changes the severity of changes observed; (3) if aged particles are less toxic; (4) if long-term low dose exposure leads to developmental and reproductive changes, and (5) if NP-exposure induced changes to the F0 generation are passed onto subsequent generations, who themselves are not exposed directly. We observed disturbances in their reproduction cycle, morphological changes, including eyes and tail defects, to each of the exposed F1-F3 generations. We also observed differences in gene expression supporting that AgNPs and TiO_2 have toxicological impacts from chronic exposure irrespective of particle aging. We were also able to see recovery in the F3 generations that had their subsequent parent generations removed from exposure.

Read-across nanoinformatics models for the assessment of NPs zeta potential based on image nanodescriptors

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The existing concern on the possible risks from nanoparticles (NPs) to human health and environment impels the development of a solid NPs risk assessment framework. Computational approaches based on experimentally measured or theoretically calculated descriptors that encode NPs structural characteristics aim to provide a significant aid to prioritize NPs for experimental testing. Recent efforts focus on the extraction of meaningful nano-descriptors directly from microscopy images, and use these image-descriptors as additional input information in predictive modelling. In the present work we introduce a novel nanoinformatics workflow for the extraction of image descriptors of NPs from TEM images using the open-source KNIME platform. Moreover, a read-across method based on the set of image descriptors and the kNN algorithm implementation was developed to assess the zeta-potential index of each of the NP included. In the proposed workflow variable selection was used to reduce the size of input parameters and highlight those that were important for interpreting how the selected nano-descriptors affect the values of zeta-potential index. The proposed model was fully-validated in accordance to the principals of OECD and afforded accurate predictions of the zeta-potential based on the different validation criteria used. That allowed us to seek for patterns and discuss similarities into the neighbourhood space, by studying the groups of the k training neighbours of each of the NPs studied and we thus propose our workflow as a reliable tool for all stakeholders interested in the prediction of NPs properties.

Adaptation of geochemical modelling tools to predict bioavailable metal concentrations in agricultural soils amended with metal oxide nanoparticles

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There is an increasing interest on the development of nano-enabled formulations for delivery of agrochemicals. To maximize benefits of nanotechnology application in agro-food production and to ensure food and feed safety, tools to predict the fate of nano-enabled agrochemicals in soil are necessary. Currently, the bioavailable concentrations of metals introduced in soils as nanoparticles need to be experimentally measured as there is no available (standard) method to predict them. Since aging is a key factor to consider in the assessment of bioavailability of nanoparticles in soils, dissolution kinetics must also be assessed. This is particularly important for agriculturally relevant metal oxide nanoparticles with slow to moderate dissolution rates in soils. In this study we developed an approach to describe the dissolution kinetics for nanoparticles in soils in combination with mechanistic complexation models for soil reactive components (i.e. ion binding advanced models for particulate and dissolved organic matter (NICA-Donnan model), the generalized two layer model for Fe/Al(hydr)oxides and a Donnan model for clay together with selected mineral equilibria) and applied these to predict the solid-solution partitioning and speciation of metal ions dissolved from CuONPs and ZnONPs in soils and to estimate the metal bioavailable pool as a function of soil type.

Subspace Clustering as a tool for the Read-Across and Categorization of Nanomaterials

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Introduction: Considerable efforts have been made to provide in silico models aimed at predicting hazards of nanomaterials (NMs). Generally, these models exploit Machine Learning (ML) techniques, including Clustering algorithms that automatically group data according to similarity in descriptors. However, as the number of descriptors increase, these similarity measures became less meaningful. Subspace Clustering is an extension of Clustering, where clusters are searched in different (possibly overlapping) combination of descriptors, called Subspaces.

Materials and methods: Our method finds overlapping clusters in data Subspaces, then it predicts an endpoint or categorizes an untested NM extracting information from NMs that appear in the same cluster(s). This method has been tested on datasets retrieved from the literature, as well as on data from EU projects. Performance has been compared to those reported in literature.

Results: Our approach was able to provide better or comparable results with respect to already published ones when applied to existing datasets or on new data.

Conclusion: Subspace Clustering have been shown to be a promising tool for both Read-Across and Categorization purposes, indeed we believe that further work can be done following this path, thus providing new insights to scientists and risk assessors.

Modelling nanoparticle transport and bio-availability in soil mesocosms

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Exposure to nanoparticles (NPs) in different soils can currently not be corrected for bioavailability, because modelling tools to do so are lacking and so are the standard tests to obtain parameters required for modelling. Existing equilibrium approaches using partitioning values have also found lacking. NP uptake and associated toxicity is therefore always calculated based on total concentrations, an approach that ignores the large differences in NP fate in different soils. The current paper explores a kinetic approach for modelling transport and bio-uptake of NPs in soils. The attachment efficiency of silver sulphide (Ag_2S) NPs was determined both via batch and column tests for a standard soil (Lufa 2.2). Different model approaches using the attachment efficiency were subsequently applied to predict Ag_2S NP transport and uptake by different invertebrate species and plants grown in soil mesocosms. While mechanistically more accurate, it is still unclear whether a kinetic paradigm can improve upon the existing equilibrium paradigm to predict bio-availability. It is likely that the many complex processes other than NP attachment for which no parameters are collected (i.e. air-water interfaces, bioturbation) play a too large role for models based on attachment only to improve upon the highly inaccurate equilibrium-based approaches.

Multigenerational exposure of Folsomia candida to copper agrochemicals: conventional and nano-pesticides

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Copper pesticides have been used for several decades in agriculture to enhance yield but recently copper nano-pesticides were introduced in the market with the intent to improve efficacy and decrease environmental negative effects benefiting from the nano specific properties. However, long term ecotoxicological induced effects of these nanopesticides on soil biota are unknown, which is crucial to derive an accurate hazard assessment. This study aimed to evaluate the multigenerational exposure of collembola to a copper conventional pesticide and a nano-pesticide, as well as the active ingredient $Cu(OH)_2$ in spiked soil. Survival and reproduction were assessed for three generations using two soil exposure setups: 1) Copper spiking only at the beginning of the multigenerational experiment; and, 2) Copper spiking at the start of each cohort. After a three generational exposure, organisms were moved to uncontaminated soil for three further generations to assess recovery. Exposure to aging soils (setup with spiking in the setup beginning) revealed an increasing tolerance across generations. In contrast, in treatments with renewed spiking, collembolan showed ongoing sensitivity. In both treatments, after being moved to clean soil, collembolans showed some recovery by displaying increased reproductive output. This study emphasises the importance of multigenerational approaches to obtain relevant evaluations of environmental risk associated with long term exposure to agrochemicals.

3D Chemical imaging with ToF-SIMS to elucidate TiO2 NPs and freshwater algae interactions

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Introduction: Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful technique that can be employed to study nano-bio interactions. The capability of detecting large molecular ions, which are formed depending on the surrounding environment of the chemical specie, distinguishes ToF-SIMS from other imaging analytical techniques. The formation and detection of these ions can provide important information regarding the distribution of the NPs in a complex system. In this study, we used ToF-SIMS analysis to investigate TiO_2 NPs and microalgae interactions.

Materials and Methods: An innovative sample preparation method was developed to preserve the pristine molecular structure, localization and identity of both TiO_2 NPs and the organic matrix. Furthermore, ToF-SIMS analysis was implemented in delayed extraction mode for the identification and localization of TiO_2 NPs in Chlorella vulgaris.

Results: The distribution and molecular surrounding of TiO_2 NPs in microalgae were acquired with a lateral resolution of 130 nm combined with a mass resolution above 5000. Highly-resolved 3D molecular images elucidated possible interaction mechanisms between NPs and microalgae.

Conclusions: The suggested sample preparation method combined with the 3D chemical visualization via ToF-SIMS paves the way for the elucidation of NPs environmental fate, related transformation processes, and exposure of aquatic organisms to NPs.

Laser desorption ionization mass spectrometry as a useful tool for nanoparticle coating characterization

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Introduction: Nanoparticle (NP) coatings are a key parameter controlling their fate in the environment. Therefore, it is of upmost importance to characterize NP coatings and to be able to track changes in particle coating characteristics. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF-MS) is a powerful technique to characterize polymers and small molecules without fragmentation using a matrix for the ionization process. Complementary information about the NP core and the coating can be obtained without the need of an additional matrix by LDI-ToF-MS. Here, we demonstrate application capabilities of LDI-ToF-MS for the characterization of organic nanoparticle coatings.

Materials and Methods: Different combinations of NP core (e.g. Au, Ag, Pt, TiO_2) and organic coating materials such as polymers and small molecules were analysed by LDI-ToF-MS.

Results: The applicability of LDI-ToF-MS depends on the type of nanoparticle coating and the concentration of coating material. Sample matrix effects may hinder straightforward interpretation of mass spectra.

Conclusion: LDI-ToF-MS is a capable and sensitive tool for the detection of organic coating on NPs. Its application for material characterization will be emphasized and its potential use to study transformation of coatings will be demonstrated.

Poster Presentations

Life span-resolved nanotoxicology in the nematode C. elegans: the gut – neural axis

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We investigate nanoparticle-bio-interactions in longitudinal studies in the model organism Caenorhabditis elegans which enables analysis of age-resolved processes and neurodegeneration. Environmentally relevant nanomaterials such as nano Ag, ZnO, CeO_2 and silica were incubated with adult worms in a 96-well plate microhabitat consisting of liquid medium and bacteria. Life span-resolved analysis identified especially middle-aged worms as vulnerable target group of nano Ag and nano silica by promoting neuromuscular defects, which normally are observed in older worms. Nano Ag induces neural beading and degeneration of the amphid neuron ADF that regulates locomotion. Notably, an accelerated increase of uncoordinated locomotion was observed resembling the rigidity in Parkinson's Disease (PD). PD is characterized by amyloid protein aggregation, which is a hallmark of neurodegenerative diseases and was also observed in intestinal cells of nano silica-exposed worms. Nano silica altered the morphology of the intestine and the protein transfer within the cells by accumulating peptides in vesicles. Thus, nano silica not only disturbs neurotransmission but also the protein metabolism in the intestine. We suggest that organ-cross talk between intestine and the neural system by neurohormone signaling may play a regulatory role in neurodegenerative nanoparticle effects.

Toxicokinetics of silver nanoparticle effects to the nematode C. elegans

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The toxicity of silver nanoparticles (AgNPs) is commonly attributed to the release of silver ions through dissolution. This study aims to investigate whether in fact ionic silver exerts observed toxicity in the nematode C. elegans. Initially, the dissolution of 50 nm AgNPs with citrate or PVP coating at their respective reproductive EC50s will be assessed at periodic intervals over the test duration. Subsequently nematodes will be exposed and transferred at said intervals into freshly made up media. Their reproductive output will be monitored under three different scenarios: 1) Media reflecting the measured dissolution dosed with $AgNO_3$ at the measured concentration of ionic silver, 2) exposures with AgNPs and added cysteine to capture free Ag^+, and 3) addition of AgNPs mimicking standard tests where a mixture of silver nanoparticles and ions will be present at the same time. These exposures will be carried out in media with different compositions and pore water extracts from soil. The comparison of the different scenarios will allow us to identify whether nanoparticle toxicity is driven by the dissolved ions or if there is a contribution of their particulate form.

Coating matters: An electrochemical based study of effect of coating in CeO_2 NPs with model membranes.

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Cerium (III) oxide nanoparticles (NPs) exist in a broad range of fields from industrial and pharmaceutical applications to consumer products. It is found in large abundance (more prevalent than gold and silver) and likely released into the aquatic environment thus prevalent in our daily lives; posing a potential hazard to our health. Therefore, it is important to study their toxicological behaviour. Studies were conducted using a high-throughput electrochemical screening platform showing membrane modifications and disruptions by a nanoparticle. This platform utilises a DOPC lipid component (that is interchangeable) as the biological cell membrane mimic. Validation with a series of nanomaterials show distinct patterns of behaviour differentiated by their physiochemical and surface functionalised properties. Two uncoated CeO_2 NPs samples of 5nm (quantum dots) and 15nm (cubes) were synthetized and characterised using DLS, TEM, EDX and diffraction patterns. No interaction was shown with lipid layers of DOPA, DOPG or DOPE. However, citrate and PVP coated samples produced a modification with DOPC layer. The findings suggest that coating may affect NPs interactions with model membranes and thus affect their activities when internalised in cells. Our findings suggest that the properties of surface functionalised CeO_2 NPs have a great influence on their lipid interaction

A Model for Competitive Adsorption in Blood Plasma and Lung Lining Fluid

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The increased use of nanoparticles (NP) and nanomaterials is pushing scientific research into trying to understand the mechanisms governing interactions between biomolecules and inorganic materials. It is known that, once a NP is in contact with a biological medium, a protein corona forms on its surface, and that the nature of the corona is what regulates the interaction between the NP and the other biomolecules. In this work we propose a method to coarse grain the interactions of inorganic nanomaterials in contact with biological fluids of arbitrary composition. Biomolecules (lipids, proteins and carbohydrates) are coarse grained by mapping their main chemical fragments onto single beads, and their interaction with the NP surface is described a potential of mean force from atomistic simulations [2]. The NP is represented by a two-layer model where the surface interacts with the molecule beads by using the beads PMF with a slab of the material, corrected by a geometric factor, while the core interacts with via van der Waals forces calculated using Lifshitz theory. This model can describe the kinetics of competitive adsorption of biomolecules on the surface of a NP. We have studied the kinetics of adsorption and the corona composition of Au NPs in a biological environment with the typical composition of lung lining fluid and blood plasma. This methodology could shed lights on the mechanisms that regulate interactions at the bionanointerface and determine the potential toxicity of the nanomaterial.

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Mechanistic insights into aluminum nanomaterial uptake and metabolism after co-exposure to vitamin A and D

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Nanomaterials (NMs) exhibit unique properties and are therefore increasingly used in a variety of different products. Nevertheless, the relationship between their physico-chemical properties and their biological activity remains unclear. A promising approach towards further elucidation is a material characterization based on the combination of different complementary techniques techniques to assess quantity, size distribution and possible induced metabolic effects in target cells. Cosmetic products contain a wide range of NMs, often in combination with complex mixtures of other chemicals. Thus, multitudes of possible enhancing or inhibiting factors are conceivable. Initial studies with Al and Al-oxide particles in combination with vitamin D have revealed individual changes in the cell membrane phospholipid pattern changes and alterations in particle uptake in the skin-related HaCaT cell line. Therefore, the uptake mechanism and distribution of both, Al oxide (Al_2O_3) and Al NMs in those cells was investigated. Furthermore, changes in the metabolic profile after treatment with these two Al species combined with vitamin A or D3 were obtained. In this context inductively coupled plasma mass spectrometry, time-of-flight secondary ion mass spectrometry and targeted metabolomics were used. Due to the combination of different methods, the changes of lipid composition and their mechanistic background can be revealed. All in all, the datasets obtained lead to conclusions about the mechanistic response after NM treatment with different additives. Moreover, an evaluation of advantages and limitations of the different techniques will be pursued.

Advanced in vitro cell culture module for long-term cultivation and toxicity screening of nanomaterials

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Introduction: Animal models are to date unavoidable necessities for toxicity evaluation of chemicals and nanomaterials. However, these models often have limitations and can be ineffective in predicting human responses. As such there is a need for advanced in vitro toxicity screening models, with maximized predictivity, improved scientific accuracy whilst reducing animal use.

Materials & Methods: Our cell culture module enables a long-term cultivation of different cell models on microfluidic chips. The chip consists of two channels, separated by a porous, cell-covered membrane allowing transport and toxicity studies of nanomaterials under dynamic conditions. In vitro models, e.g. biological barrier systems, are cultured and analyzed on a miniaturized microscope, which serves as an incubator (heating module) in parallel. Integrated electrodes on the chip quantifies the tight junction based cell barrier formation via impedance measurements.

Results: The cell models have been used for toxicity screening of nanomaterials and are suitable for dose-response analysis and the characterization of toxic effects based on physiochemical properties of nanomaterials.

Conclusion: The module is suitable for risk assessment of nanomaterials with maximized prediction of human cell toxicity. It is currently being developed into a multi-module selective targeting system mimicking tissues and organs to determine systemic effects of nanomaterials.

Human renal proximal tubule epithelial TH1 cells as in vitro kidney model

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Nanoparticles (NPs) are a promising approach for intracellular delivery of drugs, proteins, and nucleic acids, but little is known about their intracellular fate, particularly in epithelial cells, which represent a major target. The kidneys are an essential organ in homeostatic regulation and excretion of waste products from the human body, including NPs. We have chosen TH1 cells derived from primary human renal proximal tubule epithelial cells. These cells can sustain long-term proliferation in culture, exhibit contact-inhibited, anchorage- and growth factor-dependent growth and do not form tumors in nude mice. TH1 cells also perform critical metabolic activities characteristic of renal proximal tubule cells. The aim of this study was to evaluate the nephrotoxicity and capacity of NPs to cross the proximal tubule barrier. To mimic more realistic in vivo conditions, TH1 cells were seeded at the transwell membrane filter (representing the luminal compartment) while THP1 cells growing in submerged culture on the bottom part of transwell mirrored the blood cells (the peritubular compartment). Cytotoxicity of CTAB-AuNPs was determined by alamarBlue assay while the alkaline comet assay was used to evaluate the genotoxicity. In addition, fluorescent-labelled SiO_2NPs were utilized to verify the capacity of NPs to cross the proximal tubule barrier. Based on our results, TH1 cells represent a barrier which integrity was not interrupted due to treatment with tested NPs.

The challenge of detecting engineered nanomaterials in biological matrices – From sample preparation to characterization via Field-Flow Fractionation

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With the increasing production of engineered nanomaterials (ENMs) and their omnipresent applications the release of ENMs into the environment may rapidly increase with implications difficult to predict. Over the last decade various analytical methodologies for the characterization of ENMs were studied, developed and applied. However, general analytical solutions to study the fate and behavior of ENMs in the environment as well as their human- and ecotoxicological effects are very challenging and still undeveloped [1,2]. Asymmetrical Flow Field-Flow Fractionation (AF4) is a powerful separation technique that separates nanomaterials by their hydrodynamic size based on different diffusion coefficients of nanomaterials without using a stationary phase [3]. We herein present a novel strategy for a comprehensive sample preparation of ENMs-containing complex biological matrices amenable to AF4 analysis. After spiking the respective samples with different ENMs an alkaline solubilization step was performed based on a procedure published by Bolea et al. [4]. For the subsequent analysis of such prepared samples, AF4 was hyphenated with a multi-detector approach. This preparation procedure followed by AF4 analysis was successfully established for polystyrene, silver and titanium dioxide nanoparticles offering a promising analytical methodology for the investigation of the fate and behavior of ENMs in complex biological matrices.

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The intrinsic metal content of individual A549 cells as a baseline for cellular exposure to metal nanoparticles

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Metal nanoparticle uptake by cells is an important process that may contribute to metal bioaccumulation and toxicity. Metals are present in the cellular environment as ions, albeit in quantities that are not well studied, especially at individual cell level, due to lack of appropriate instrumentation that would enable measurements of metal content easily and reproducibly. Here we introduce a new analytical method, Single Cell ICP-MS (SC-ICP-MS), which we use to investigate the variability in intrinsic metal concentrations of A549 cells, for a range of metals with different biological roles. Ni, Mn and Co metal content within cells were measured as these essential nutrients could potentially be used to quantify the cell number. Each showed a consistent presence within the A549 cells analysed, but little to no presence in DEM media, Trypsin or ultrapure water, which strongly suggests that the metals detected were intrinsic to the cells and can thus act as cellular markers for cell number quantification. Cell concentrations of Ni and Co were very similar, suggesting the method is indeed measuring the cellular concentration. This presents a new method of cell elemental composition determination and cell counting, providing a robust basis for subsequent quantification on nanoparticle uptake and load per individual cell.

Optimizing concentration of biological media at different temperatures for silica nanoparticle stability

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Background: Nanoparticles (NPs) have numerous applications in biomedical research and thus a thorough understanding of their interactions and toxicity to cells using in vitro and in vivo assays is particularly important. The presence of proteins, electrolytes, and lipids in biological media can critically affect the functional physio-chemical properties of NPs. Stabilizing NPs in biological media has been challenging, particularly in terms of controlling particle size and monodispersity. Functionalising NPs surface with polymers such as PEG, PVP, and CTAP has been used traditionally to minimise protein attachment and steric bonding and thus prevent NP aggregation and agglomeration.

Objective: To vary maintenance medium (DMEM, from EpiSkin™) concentrations for NP stability in cell media.

Method: Ultrafine silica NPs (SiNPs ≈ 2 nm), produced using the lysine-silica synthesis protocol, were filtered (0.22 µm) before x100 dilution with ultrapure water (UPW). The sample was added in cell media at varying UPW concentrations by volume (mL) and kept at room temperature (19-25°C) or in an incubator (37°C) for several hours. Dynamic Light Scattering (DLS) was carried out to monitor particle size and size distribution in the media; zeta potential was also monitored by DLS whereas particle size was also assessed by Transmission Electron Microscopy.

Result: In the initial suspension, SiNPs were stable, as indicated by zeta potential (-33.5±6.8 mV). When introduced to UPW, NPs showed an increase in size, but

remained stable showing high zeta potential (-30.1 mV); the DLS results further showed some aggregation in the form of a second peak at around 60-80 nm. From the media tested, optimal stability was observed at volume (mL) ratios of SiNP:DMEM:UPW 1:1:5 and 1:1:10 at room temperature and at 1:1:5 in the incubator (where SiNP is the initial suspension). In all conditions, increase in particle size could not be completely avoided, most likely due to adsorption of proteins on NP surfaces, and further formation of aggregates in the medium in all conditions.

Conclusion: SiNPs were stabilized by minimizing the amount of protein, electrolytes and lipids in cell media, although some size changes were inevitable, in the media tested.

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Effective and reliable screening techniques for nanosafety assessment are essential to identify nanomaterial hazards. Existing toxicity screening methods require a high level of user skill, limiting their wider potential use. To improve nanosafety assessment procedures, a novel microfluidic screening device has been designed that enables nanomaterial hazards to be identified rapidly with minimal user knowledge using an electrochemical measurement on a lipid coated electrode. The main features of the design include fluid reservoirs to store buffer, lipid and nanomaterial samples; a microfluidic flow cell containing a sensor element on a fabricated electrode for electrochemical analysis and pumps used to control the flow of fluid. Automation of the pumps enabled accurate repeatability of the screening process through precise control of the fluid flow rates and significantly reduced the required skill level of the operator, with the system requiring minimal input on a user-friendly visual control interface to operate. To demonstrate the performance of the system, various nanomaterial samples were screened using the device. Samples were injected into the flow cell to assess their interaction with the lipid coated electrode through analysis of a cyclic voltammetry response, showing good agreement with results obtained using existing test methodologies.

Enhancing the use of in vitro (neutrophil) and zebrafish embryo models as alternatives to rodent testing for assessing immunological responses to nanomaterials (NMs)

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Nanomaterials (NMs) have been shown to induce various biological responses such as inflammation, oxidative stress, cytotoxicity and genotoxicity. The toxicity of NMs is highly dependent on their physicochemical characteristics such as size, shape, composition, and charge. Current testing strategies for the safety assessment of NMs commonly involve investigation of oxidative stress and inflammation using *in vitro* cell-based assays and *in vivo* rodent studies. There is a huge diversity of NMs whose safety needs to be assessed, and there is a need to move away from using *in vivo* models as they are expensive, lengthy, and their use is associated with ethical implications. The 3Rs principles promote the Refinement, Reduction and Replacement of animal testing within scientific research. In order to align nanotoxicology testing with the 3Rs, an alternative testing strategy using non-rodent models must be sought. Zebrafish embryos and larvae (up to 5 days post-fertilisation) are not protected by UK or EU legislation, making them an ideal *in vivo* alternative to rodents. Our work aims to investigate the suitability of using the transgenic zebrafish embryo model as an *in vivo* non-rodent replacement within nanotoxicology. More specifically, a transgenic strain with fluorescent neutrophils (Tg (mpx:EGFP)) will be used to investigate inflammatory responses to NMs over time. Neutrophils are the first cells to be recruited to inflammatory sites where they act to neutralise pathogens and clear infection. The failure of inflammation to resolve and the persistence of neutrophils at inflammatory sites can lead to chronic inflammation. Chronic inflammation is associated with a variety of diseases, and thus it is vital to determine the inflammatory effect of NMs. The neutrophil response to NMs will also be investigated *in vitro* via assessment of chemotaxis, cytokine production, activation of respiratory burst, and NETosis using primary human neutrophils and the HL60 cell line. Our pilot work indicates that primary human neutrophils perform well *in vitro*, with these cells responding to chemotactic agents in cell migration assays. We are continuing to optimise this assay for use with HL60 cells. In our pilot studies with the transgenic zebrafish larvae, we have

utilised the established ‘tail fin injury’ model to create an immunocompromised model of inflammation. This work indicates that the inflammatory response can be induced via tail injury in the larvae, and that this response may be enhanced or prolonged by aqueous exposure to chemoattractants post-injury. We will use this model to investigate the inflammatory response of immunocompromised larvae to NMs.

Are existing standard methods suitable for the evaluation of nanomedicines: some case studies.

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The use of nanotechnology in medical products has been demonstrated at laboratory scale, and many resulting nanomedicines are in the translational phase towards clinical applications, with global market trends indicating a strong growth of the sector in coming years. The translation of nanomedicines towards the clinic and subsequent commercialisation may require the development of new or adaptation of existing standards to ensure the quality, safety, and efficacy of such products. This work addresses some identified needs, and illustrates the shortcomings of currently used standardised methods when applied to medical-nanoparticles to assess particle size, drug loading, drug release and in vitro safety. Alternative physicochemical and in vitro toxicology methods, with potential to qualify as future standards supporting the evaluation of nanomedicine, are provided.

The development of a guidance protocol for selection of the most appropriate and effective methods for detecting reactive oxygen species and oxidative stress in response to nanomaterials

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Reactive oxygen species (ROS), induced directly or indirectly by nanomaterials (NMs), and disruption of redox homeostasis are widely reported to account for the toxicity of many NMs. Therefore numerous acellular and cellular assays have been developed, and are routinely used to assess ROS generation and their consequences upon biological systems. However, the literature on NM-induced ROS generation can at times be confounding, which may be due to limitations of specific assays, from assay interference by NM characteristics, or from selection of limited or inappropriate assays. Under the grouping, read-across and classification framework for regulatory risk assessment of manufactured nanomaterials and safer design of nano-enabled products (GRACIOUS), we aim to assess the current analytical techniques and methodologies with emphasis on limitations, reliability and accessibility as well as including recent results from comparative approaches. In this study we aim to collate valuable information about current analytical methodologies and their outputs, to devise a sequence and strategy to allow effective testing of NM-induced ROS generation and consequence; this may provide a standard operating procedure which can be used for read-across purposes in the assessment of NM-induced effects relating to ROS.

ACEnano Knowledge Warehouse to support documentation and collection of nanomaterials physicochemical characterisation data

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ACEnano (Horizon 2020 Project no. 720952) aims to introduce confidence, adaptability and clarity into nanomaterial risk assessment by developing a widely implementable and robust tiered approach to nanomaterials physicochemical characterisation. The knowledge warehouse (KW) supports these activities related to data collection and methodology optimisation, and aims to further disseminate this knowledge to the nanosafety community in a re-usable format. The KW includes multiple instances (protocols, data and dissemination) to optimally accommodate the requirements of the different data types (e.g. raw, processed data and protocols). The protocols database facilitates the addition and sharing of methods in a questionnaire-like format that guides the user through the documentation process from sample identification and description to the preparation and measurement. The structured protocols allow for an easier comparison of the experimental design and better comparability of results. Further, the data warehouse offers long-term storage of the results that are directly linked to the methods applied. Therefore, the ACEnano KW provides a central place to access harmonised and standardised methods applied for physicochemical characterisation of nanomaterials, supporting the implementation of Findable, Accessible, Interoperable and Reusable (FAIR) data principles, the reproducibility and documentation process towards the goal of generating reference resources for nanomaterials risk assessment.

Read-across in silico investigation of the bioactivity and toxicity behaviour of carbon nanotubes

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The extended use of carbon nanotubes (CNTs) in various applications has raised concerns on the impact that these materials may have on the living organisms and the environment, thus emphasizing the urgent need for further toxicological investigation. In order to avoid expensive and time-consuming experimental practices, a significant number of alternative novel techniques, such as Quantitative Nanostructure Activity Relationship (QNAR) models for the biological and the toxicity assessment of different types of nano-structures, has been proposed in literature. In the presented work, a validated QNAR workflow for the biological and toxicity assessment of decorated multi-walled CNTs is proposed. A classification kNN model for the prediction of protein binding and toxicity of CNTs based on calculated molecular descriptors of CNTs' surface ligands was developed using KNIME platform. Using external validation tests high predicting accuracy was achieved both for the biological as well as the toxicity endpoint. With respect to the read-across framework, the groups of similar neighbours for each CNT included in the test set, were defined. Special focus was given on the investigation of those properties that significantly affect the two investigated endpoints, in order to get a better insight of the structural characteristics that control the CNTs behaviour. It is demonstrated that the presented tool can simplify the design and screening of novel CNTs, and thus can be a useful aid for the decision making of all interested stakeholders.

Making use of available and future data to predict the properties, interactions and hazards of engineered nanomaterials by means of in silico tools: a critical review

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Introduction: The heterogeneity of nanomaterials (NMs), with respect to physicochemical properties and observed effects, has made their case-by-case risk assessment unsustainable in terms of costs, time, and number of animals involved in testing. Thus, in silico methods such as (Q)SAR/(Q)SPR, Grouping and Read-Across have become fundamental to assist decision making related to the safety assessment of NMs.

Materials and methods: A number of studies have been selected from the literature and evaluated against a set of criteria based on the OECD principles for the validation of (Q)SAR models.

Results: Even though modelling techniques, model descriptors, modelled endpoint, and a scientifically sound mechanistic interpretation have been in general well communicated, the predictive ability of the models often resulted to be not properly evaluated. Moreover, Applicability Domains have not always been assessed, and the evaluation of the robustness, uncertainty and sensitivity of the models is insufficient.

Conclusion: Attention should be paid in properly validating models and in evaluating model performances, to provide more predictive and reliable models. Future work will focus on exploring novel Machine Learning methods, whilst currently developed models should be tested and possibly validated based on existing and newly generated data.

Cytotoxicity of Silver Nanoparticles: Zebrafish cells a new experimental model to evaluate nanoparticles' toxicity.

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The study of the behaviour and interactions between nanomaterials, environment, and organisms is a rapid growing field. Nevertheless, there still exist gaps that need to be addressed, including the necessity to evaluate the potential environmental risks of nanomaterials related products. Silver nanoparticles (AgNPs) have been used in a variety of cytotoxicity and genotoxicity studies, nonetheless there is still an understood gap regarding their cytotoxic effects on aquatic organisms. Here, the use of fish cell lines, as a proxy for the organism, may represent an economical and effective model to study nanoparticle behaviour and effects on aquatic biota. Cell culture involves higher control in the experimental conditions; targeted and accurate results make it an effective tool to assess the possible toxicity outcomes without sacrificing animals as in in vivo studies. The current study is aimed to study cytotoxicity-mediated changes induced by silver nanoparticles, providing key results to understand the nanoparticles' effects in an early stage for aquatic organisms, including possible outcomes related to their dissolution, aggregation, uptake and bimolecular cell death responses. Providing a baseline for AgNPs-mediated cytotoxic assessment in zebrafish cells for the first time.

Sulfidized silver nanoparticles induce lower toxicity than pristine ones to the pond snail Physa acuta

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Due to their widely use in antibacterial products, silver nanoparticles (AgNPs) end up in wastewater treatment plants (WWTPs) being discharged into aquatic environment. In WWTPs, AgNPs undergo physical-chemical transformations, which modify their properties. It is crucial to consider such transformations when assessing their impacts to aquatic organisms. The freshwater snail Physa acuta was used to evaluate potential toxic effects of pristine and sulfidized AgNPs, which simulate the transformation underwent in WWTPs. Acute tests with juveniles and adult snails were performed to assess lethality. Chronic tests were performed to assess effects on survival, reproductive success (number of egg clutches and eggs per clutch) and shell length. Acute toxicity of pristine AgNPs was higher in juvenile snails than in adults. Ag_2S NPs caused no acute toxicity at 0.1-10 mg/L. Pristine AgNPs caused a decrease in the number of egg clutches with increasing concentrations, while Ag_2SNPs caused the inverse effect. During chronic exposure, higher mortality was observed for Ag_2SNP exposure, with no observed effects on the number of eggs per clutch or shell length. This study highlights the differential toxicity between the two Ag particulated forms, showing that understanding fate and exposure in the environment is essential to derive accurately environmental hazard.

The influence of differing soil properties on the uptake of different Ag nanoparticle forms from soil by plants exposed from seed.

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In order to develop models that support site-specific risk assessment for engineered nanoparticles (ENPs), a better understanding of how ENP transformation processes, fate and bioavailability are influenced by soil properties is needed. The aim of this study is to investigate the uptake of Ag from different Ag forms (Ag0 ENM, Ag_2S ENM and $AgNO_3$) in wheat, Triticum aestivum, from three different natural soils with varying pH (5.5-7) and organic matter content (2-16%). Plants were exposed from seed in spiked soils (10 mg Ag/kg, nominal concentration) for 23 days, post-emergence. Plants were sampled 1, 4, 6, 13 and 23 days post-emergence. Ag concentration in the plant roots and shoots were measured. Different uptake was observed from the different soils, with the highest uptake rates and bioaccumulation from the soil with the highest pH (6.9) and lowest organic matter content (2%). Plant roots accounted for most Ag accumulation with minimal transfer the shoots. Soil pore water was extracted (0.45µm and 10 kDa filtered) from each soil Ag treatment as a measure of Ag bioavailability in the soils. Pore water concentrations are expected to better predict uptake into the roots with the rhizosphere environment influencing Ag form and uptake potential.

Comparison of the toxicity and bioaccumulation of different types of Cd-based Quantum Dots for model plants

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The quantum dots (QDs) are fluorescent semiconductor nanocrystals which are being extensively developed because of their unique size-dependent optical and photophysical properties. Unfortunately, several disadvantages in the using of QDs occurred. Especially, the leach of free Cd ions from core Cd-based QDs in the contact with aqueous media is their biggest drawback. However, it is believed that the encapsulation of nanoparticles should reduce their toxicity and increase their stability in different environments. We report on the toxicity and bioaccumulation of different types of Cd-based QDs for a selected model plant organism. After a certain exposure, we monitored the growth of roots and dry biomass of roots as a macroscopic toxicity end-point. Then we determined the total Cd content in different plant parts by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Consequently, the plant roots were inspected by the fluorescence microscopy to determine if Cd-based QDs are adsorbed on the root surface and how it depends on the QD type. Selected QDs showed different toxicity in correspondence with different Cd bioaccumulation patterns. We discussed possible hazardous effects of Cd-based QDs for selected plant as well as the environmental implications of the exposure to Cd-based QDs.

Determination of spatial distribution of selected lanthanides contained in upconverting nanoparticles in plant tissues by laser induced breakdown spectroscopy

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In the past few years, lanthanide-doped nanoparticles - upconverting nanoparticles (UPCNs) have attached much interest as a new possibility of luminescent optical labels for many biological applications due to their unique properties, especially high tissue penetration depth, a potential for lower toxicity and the possibility of photon upconversion which is important for target imaging techniques. Concerns about their toxicity are still the main issue for applications. Their small size is potentially harmful to allowing deep tissue penetration and accumulation there. Our study is focused on the spatial distribution of lanthanide into different plant tissues of the model plant in the aquatic environment compared to lanthanide salts. Commonly used toxicology endpoints, such as root growth or total length of the root system were used to evaluate the toxicity effect. Cross-sections from different plant tissues were prepared for monitoring of depth distribution of UPCNs. The spatial distribution of selected lanthanides was established by Laser-Induced Breakdown Spectroscopy (LIBS) with tens of micrometer scale which is a suitable resolution for first monitoring study. The study provides information about the underlying mechanism of water-soluble UPCNs, dispersed in aqueous medium, uptakes by model plants. We also discussed possible risk assessment and toxicity of UPCNs.

Shape Dependent Transformation and Translocation of Ceria Nanoparticles in Plant

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The association of physicochemical properties of CeO_2-NPs (CeO_2 nanoparticles) per se with their transformation is not well understood. This study for the first time compared the translocation and transformation of octahedral, cubic, rod, and irregularly shaped CeO_2-NPs in hydroponic cucumber plants. Cerium contents in roots were close between different treatments, while the largest amount (153 mg/kg) of Ce accumulated in rod-like CeO_2-NP treatments. Transmission electron microscopy and X-ray absorption near edge spectroscopy show that rod CeO_2-NPs transformed faster and more than other CeO_2-NPs, with nearly 40% of Ce in the form of Ce(III) species in roots ($CePO_4$) and shoots (Ce carboxylates). Rod-like CeO_2-NPs transformed to a degree greater than those of the other CeO_2-NPs in solution simulating the plant exudates, indicating that rod-like CeO_2-NPs have the highest chemical reactivity. These results suggest that the intrinsically different chemical reactivity of differently shaped CeO_2-NPs resulted in their different transformation and translocation capacities in plants. This study provides new insight into plant–NP interaction, highlighting the significance of the shape of nanoparticles in assessing their environmental behavior and impacts. We suggest that the influence of shape should be also considered for other nanomaterials and systems in developing an accurate understanding of the nano–bio interactions

Microscopy methods for assessing the biological uptake and effects of ENP

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Manufactured nanomaterials are of great interest for biomedical science, due to emerging importance in diagnosis and treatment of disease as well as increasing concerns regarding the potential for inadvertent environmental exposure and possible toxicity. Thus potential for cellular internalization and effects is of utmost importance in both cases. Microscopy is an established method for characterization of ENP and ENP-cell interactions, the use of fluorescent labelling in conjunction with reflectance detection of unlabelled NMs provides a host of opportunities for investigating cellular interactions and effects. We have utilized automated long-term live imaging, correlative confocal fluorescence and reflectance, superresolution reflectance and traditional TEM to probe the uptake and subcellular localization from cell samples exposed to a host of different NMs including TiO_2, Au and Ag size ranges (10 – 200nm), Fe_xO_x and CeO_2. Fully automated analysis routines were developed to enable the quantification of ENP presence within cells via the different methodologies. Reflectance techniques provide important tools for the future assessment of the efficacy and safety of ENPs for clinical use, enabling quantitative analysis of uptake route, subcellular localization and ENP intracellular fate.

New insights into the interaction of GMs with bacteria film

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Graphene based material (GMs) have been reported to exhibit antibacterial activity; however, impact of GMs on bacteria biofilm has not been well understood. Previous studies regarding the GNMs and bacteria were mostly performed in in saline or minimal medium solution; however, toxicity and behavior of GMs in rich medium are not well understood yet. In fact, the scenario of bacteria in rich medium do exist, e.g. in urban wastewater. We compared the impact of graphene oxide (GO) and reduced graphene oxide (rGO) on biofilm formation and development in both Luria-Bertani (LB) medium using Escherichia coli and Staphylococcus aureus as models. GO significantly enhanced the cell growth, biofilm formation, and biofilm development even up to a concentration of 500 mg/L; while rGO (≥50 mg/L) strongly inhibited cell growth and biofilm formation. However, the inhibitory effects of rGO (50 mg/L and 100 mg/L) were attenuated in the mature phase (>24 h) and eliminated at 48 h. GO at 250 mg/L decreased the reactive oxygen species (ROS) levels in biofilm and extracellular region at mature phase. ROS levels were significantly increased by rGO at early phase, while they returned to the same levels as control at mature phase. These results suggest that oxidative stress contributed to the inhibitory effect of rGO on bacterial biofilm. We further found that supplement of extracellular polymeric substances (EPS) in the growth medium attenuated the inhibitory effect of rGO on the growth of developed biofilm. XPS results showed that rGO were oxidized to GO which can enhance the bacterial growth. We deduced that the elimination of the toxicity of rGO at mature phase was contributed by EPS protection and the oxidation of rGO. This study provides new insights into the interaction of GMs with bacteria biofilm.

Effect of electric current and zero-valent iron on bacterial consortia in site polluted by chlorinated ethenes

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Chlorinated ethenes (CEs) are worldwide groundwater contaminants and difficult to remedieate. Combining microscale zero-valent iron (mZVI) and direct electric current (DC) is very promising approach for a successful CE remediation, where the contaminant concentration is too high. When the CE concentration decreases, microbial degradation could be more efficient. Here we present the effects of DC and mZVI on indigenous microorganisms (MOs) in a Spochemie site in the Czech Republic. Three groundwater wells were sampled during a 300-day study, namely cathode well, anode well and reference well. The cathode and anode was switched 140 days after mZVI application. The effects of the DC on MOs were studied on: (1) total cultivable aerobic and anaerobic bacteria by colony-forming unit (CFU); (2) total bacteria (16S rRNA gene) by U16SRT marker and organohalide-respiring bacteria be markers for vcrA: Dehalococcoides sp., DHC-RT: Dehalococcoides sp. (16S rRNA gene), and Dsb: Desulfitobacterium (16S rRNA gene) using quantitative polymerase chain reaction (qPCR); (3) bacterial communities by targeting the V4 region of 16S rDNA gene and Next-generation sequencing (NGS). The physico-chemical parameters: pH, oxidation-reduction potential (ORP) and degradation products of CEs were also monitored. The significant change was found after switching DC at only monitoring cathode well including the total bacteria increased from 140 until 173 days, and Dehalococcoides and Dehalococcoides as well as Desulfitobacterium also increased during 300 days. The cultivable bacteria in both aerobic and anaerobic conditions changed only marginally. Interestingly, bacterial communities at genus level in the cathode well dynamically increased: Desulfatiglans (140 days), Ferribacterium and Arcobacter (201 days) and other bacteria (300 days), whilst genera Gandidatus_Falkowbacteria and Commamonadaceae notably increased in the anode well. The reference well

maintained very similar diversity during 300 days. The switched anode-cathode regime turned cathode well to neutral and aerobic condition, which was a more favourable for bacterial growth, while the opposite prevailed in the anode well. Importantly, the degradation products of CEs increased in agreement with the other parameters. We found that the combined mZVI - DC approach showed positive effects on the CE remediation and bacteria were able to proliferate, especially those involved in biodegradation of vinyl chloride.

A comparison of analytical techniques for measuring the attachment rate of nanomaterials to soil in kinetic batch tests.

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The attachment efficiency (α) is regarded as a key fate descriptor for model predictions of engineered nanomaterial (ENM) mobility in natural soils. While saturated column experiments are the most common methods for measuring the attachment efficiency, kinetic batch tests may provide a simpler alternative approach. Previous experimental developments have led to successful estimations of α for ENM attachment to synthetic solid media, but development of approaches to quantify attachment to environmental media such as soils is still in its early stages. Here, we present a kinetic batch test method for measuring α for natural soil samples, using 80 nm and 20 nm citrate coated gold nanoparticles. The method involves measurement of the change in the unattached ENM concentration in a suspension of soil particles over a set reaction time. Artificial rainwater and saturated porewater solutions were used as the background matrices, with the ratio of soil to ENM manipulated to allow for accurate quantification of unattached ENM concentrations over the experimental timescale. Concentrations of unattached ENMs were obtained through UV-Vis spectroscopy and single particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS) and compared. Preliminary results using UV-Vis spectroscopy suggested an inadequately small window of workable nanomaterial/soil concentration ratios that could be used to obtain α. Further, a high, variable DOC signal from the soil prevented reliable background corrections and increased ENM detection limits. In contrast, spICP-MS allowed for a wider range of nanoparticle and soil concentration ratios which resulted in measurable unattached concentrations of nanomaterials. Low attachment efficiencies were observed for the initial soil to ENM ratios, which was also observed in saturated column tests. Further developments with different soils and ENM types are ongoing.

List of Attendees: