2nd NANOSAFETY FORUM FOR YOUNG SCIENTISTS
Visby, Gotland, Sweden
15-16th September, 2016

OPENING KEYNOTE LECTURE

Nanosafety Research: Lessons and Future Challenges

Iseult Lynch
Department of Earth and Environmental Sciences, School of Geography, University of Birmingham, Birmingham, UK
e-mail: I.Lynch@bham.ac.uk

EC investment in nanosafety during FP7 exceeded €200M, with 50 projects funded directly via NMP, Life+, Marie Curie, Research Infrastructures and others. Together, these projects represented 27,000 person-months (2,250 years) of effort (most of which is now completed, with the last FP7 projects ending in 2017). The 50 nanosafety projects comprised 758 partner organisations, with 390 unique organisations (many institutes were involved in several projects and/or follow-on projects). 135 of these were enterprise partners (96 classified as SMEs), representing 28% of the total partnerships (SMEs 19%) and 22% of the EC financial contribution (SME beneficiaries 18%).

The 50 Nanosafety projects spanned from nanomaterials synthesis and characterisation, including in complex matrices and products, through exposure and life cycle considerations to toxicity and ecotoxicity assessment and finally risk assessment and regulation. The last category is quite broadly defined as projects related to standardisation and validation, as well as ontology and databases are included here, along with the large NanoReg project consisting of EU FP7 and member state funding along with contributions from industry, via a public-private partnership arrangement. Overall, the 50 projects represent a pretty-well balanced portfolio, although ecotoxicity assessment has tended to be under-emphasized to date (often being 1 WP in large toxicity-focussed projects).

The review of the progress of FP7 projects identified as series of major scientific challenges in the next five years including the need to demonstrate at least some 'safer by design' nanomaterial prototypes, the need to focus testing on industrially relevant materials (which may not disperse ideally, and are likely highly polydisperse in size and other properties), and ultimately to translate the scientific advances into utilisable low cost assays and *in silico* approaches that can be utilised for regulatory testing. Central to this is agreed data standards and enhanced research infrastructure for nanoinformatics. Linked into this could be an increased focus on benign-by-design approaches which consider aspects of recovery, re-use and recycling of the nanomaterials components at the end of their life cycle. Given the critical shortage of many key resources, use of nanomaterials both for recovery of critical resources, and strategies to recycle nanomaterials themselves would align with EU research priorities and the circular economy well. Closer integration of researchers addressing nanosafety, nanomedicine, pilot lines, critical resources and the circular economy would facilitate a more rapid translation of nanosafety progress into practice, which would be facilitated by a greater focus on nano-governance and responsible research and innovation.

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CLOSING KEYNOTE LECTURE

Small Meets Smaller: Nanomaterial-Microbe Cross-Talk - Physico-Chemical Principles and (Patho)Biological Consequences

Roland H. Stauber
Department of Nanobiomedicine, University Medical Center of Mainz, 55101 Mainz, Germany
e-mail: rstauber@uni-mainz.de

Pathogenic microorganism can cause severe diseases. Also, scientific and medical interest in the human microbiome, defined as the sum of all microbial organisms residing inside the body, has increased dramatically. Notably, the infection paths of pathogenic microorganism overlap with major entry routes for nanoparticles (NPs), occurring during environmental exposure or deliberate medical applications. For example, besides NPs, the air we breathe is also filled with a high number of fungal spores, originating from a variety of fungal species. Hence, it is surprising that the interaction of NPs with (pathogenic) microorganism and its (patho)biological consequences have not yet been investigated in detail.

As the physico-chemical characteristics of NPs (co)define their behaviors and (patho)biological activity in physiological systems, we studied a library of various model NPs widely varying in size, material, shape, and surface functionalization. The interaction of NPs with different microorganisms as well as the impact of NPs on microorganism-host cell responses was investigated by comprehensive analytical approaches.

We report how different microorganisms interact with NPs, discuss the underlying physico-chemical principles, and demonstrate how these interactions can impact the (patho)biological outcome and fate of exposure of the human host to both, NPs and microorganisms. We expect that the identified mechanism will be of biomedical and toxicological relevance for the field.

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ABSTRACTS OF ORAL PRESENTATIONS

1

Development and Refinement of Microscopic and Spectroscopic Techniques for the Detection and Characterization of Manufactured Nanomaterials

Steinhoff B., Schönherr H.
University of Siegen, Physical Chemistry I, Siegen, Germany

Background: Due to their ever-expanding application fields in everyday life, manufactured nanomaterials (MNMs) are omnipresent in the environment. Among those, silver and titanium dioxide nanoparticles (NPs) represent two examples, which are very widespread, but whose end-of-life impact on the environment remains unclear. MNMs are subject to physico-chemical changes like the adsorption of biomolecules, especially during wastewater treatment. This alteration in NP properties may lead to an increase in bioavailability and toxicity for water organisms. Therefore, analytical methods have to be optimized with known samples to study the characteristics of MNMs in field samples within the framework of the project FENOMENO (Fate and effect of wastewater-borne manufactured nanomaterials in aquatic ecosystems).
Materials and Methods: Ag (NM-300K) and TiO_2 (NM-105) NPs were investigated via Darkfield Optical Microscopy for overview scans and Transmission Electron Microscopy as well as Atomic Force Microscopy for NP size and shape determination. The latter technique was also used to study the mechanical properties of the NP corona, whose chemical composition was determined by X-Ray Photoelectron Spectroscopy (XPS).
Results: It was found that pristine Ag NPs were mostly covered with stabilising agents that were hence removed via oxidative and thermal treatment to reveal the nature of the metallic NPs. A more prominent silver signal in the XPS spectra revealed that the dispersants were partially removed which was verified by subsequent AFM analysis.
Conclusions: The approaches described above are useful methods for the characterization of metallic NPs and their adsorbates in biological samples but still need to be further refined.

2

Flow Field-Flow Fractionation-Based Approach as Analytical Tool for Ag Nanomaterial Design

Marassi V.[1], Di Cristo L.[2], Casolari S.[1], Costa A.L.[3], Prina-Mello A.[2], Reschiglian P.[1]
[1]University of Bologna, Chemistry, Bologna, Italy, [2]Trinity College Dublin, Trinity Translational Medicine Institute (TTMI), School of Medicine, Dublin, Ireland, [3]CNR - ISTEC, Nanotechnology, Faenza, Italy

Background: The access to suitable analytical tools for characterisation of silver nanoparticles (AgNPs) is of primary importance, given their rising employment in technological and biological relevant applications. It is known that AgNPs undergo changes in composition, size, shape and core-shell properties when in the exposure medium. These modifications influence the overall toxicity and can cause adverse effects upon exposure.
Current characterization techniques like transmission electron microscopy (TEM) and dynamic light scattering (DLS) present various limitations: the introduction of an in-flow separation technique as characterisation step provides reliable data regarding samples in suspension and collectable fractions to be individually characterised/tested.
Material and methods: The aim of this work is to assess the colloidal properties of differently coated silver nanoparticles (AgNPs) when dispersed in aqueous media. By exploiting the coupling of a soft separation technique (hollow fiber flow field flow fractionation, HF5) with various detectors online and offline (fractionation characterisation), we determined their size, shape, surface charge and ionic release. The non-destructive method applied, allowed for the separation of different phases (ions, nanoparticulates) that could be therefore tested individually to investigate their specific properties.
Results: In this study a dataset based on the physicochemical properties of differently coated AgNPs is presented, and related to their toxic and antiseptic effects, to provide the selection criteria for ranking the best performing material for healthcare applications
Conclusions: Outcomes can be the introduction of HF5-based characterization as part of protocols to investigate the component-specific hazard of nanoparticles suspended in aqueous media.

3
Superhydrophilic anti-Fouling Electrospun Cellulose Acetate Membranes Coated with Chitin Nanocrystals for Water Filtration

Goetz L.A.[1], Jalvo B.[2], Rosal R.[2], Mathew A.P.[1]
[1]Luleå University of Technology, Division of Materials Science, Luleå, Sweden, [2]University of Alcalá de Henares, Department of Chemical Engineering, Alcalá de Henares, Madrid, Spain

Background: Pressure-driven liquid filtration using electrospun membranes are challenging due to limitations related to mechanical strength, chemical and thermal stability and biofouling.
The current study presents a new approach to functionalize electrospun cellulose acetate (CA) via impregnating the electrospun fibers with chitin nanocrystals (ChNC). These nanocrystals have high surface area, good mechanical properties and also antifungal and antibacterial properties, so they are good candidates to create a new generation of membranes for water purification applications.
Material and Methods: ChNC were prepared via HCl acid hydrolysis. Electrospun was prepared with cellulose acetate with a supplied voltage of 9 kV. Impregnated membranes (CA-ChNC) were prepared via Buchner funnel filtration apparatus. The surface morphology of the membrane was examined using scanning electron microscopy (SEM) and atomic force microscopy (AFM). Tensile tests were performed on mats using a universal testing machine. Flux tests were performed by filtering distilled water through the membranes. Surface wettability tests were carried out using contact angle meter. Biofilm assays were tested using different fluorescence techniques.
Results: Coating of CA random mats using 5% ChCN increased the strength by 131% and stiffness by 340% accompanied with a decrease in strain. The flux through these membranes was also higher than in control mats. Besides, ChCN produced superhydrophilic membranes (contact angle 0°) from the original hydrophobic CA mats (contact angle 136°). CA-ChNC membranes also showed significant reduction in biofouling and biofilm formation.
Conclusions: CA-ChNC mats resulted in high flux membranes which shows potential in future water ultrafiltration applications based on 'green nanotechnology'.

4
Common Pitfalls when Preparing Nanoparticle Dispersions - Influence of Delivered Acoustic Energy and Sonication Method on Sedimentation and Dissolution Rates

Hedberg J.[1], Pradhan S.[1], Blomberg E.[1], Wold S.[2], Odnevall Wallinder I.[1]
[1]KTH Royal Institute of Technology, School of Chemical Science and Engineering, Surface and Corrosion Science, Stockholm, Sweden, [2]KTH Royal Institute of Technology, School of Chemical Science and Engineering, Applied Physical Chemistry, Stockholm, Sweden

Background: An important aspect in experiments with metal nanoparticles (MNPs) is to have a robust and reproducible dispersion method. If not, there is an evident risk that agglomerates of different sizes are present in the start solutions and differences in agglomeration and sedimentation dynamics in the systems.
Material and methods: Copper, manganese, zinc oxide, and aluminum NPs were studied. These MNPs were sonicated using different methods and with different delivered acoustic energies. The particle stability was monitored by determining size distributions using DLS. AAS was used to measure the release of metals from the MNPs. DLVO calculations were performed to predict the aggregation behavior of the particles in solution.
Results: Delivered metal concentrations when pipetting from the stock solutions to the samples were significantly lower (30-80%) than the nominal concentrations. Agglomeration and sedimentation rates were rapid even at high delivered acoustic energies. Observed results correlated with DLVO theory, which showed attractive forces between the NPs, leading to agglomeration. The metal release was 0.1-1.6 % of the total amount of MNPs when sonicating in water. When adding BSA as a stabilizing agent, the release was higher (up to 4%). The effect of sonication on the zeta potential of the MNPs was negligible.
Conclusions: The transferred dose of MNPs from stock solution, the dissolved fraction after sonication, the impact on zeta potential, agglomeration, and stability in solution should be considered in e.g. toxicology investigations. If neglected, there is a high risk of conclusions based on artefacts originating from the sonication procedure.

5

Multi-Readout *in Vitro* High-Throughput Screening Analyses Serve Efficiently for Evaluation and Ranking of Nanomaterial Toxicity Under Diverse Testing Protocols

Hongisto V.[1], Nymark P.[2], Hattara J.[1], Kohonen P.[1,2], Grafström R.[1,2]
[1]Misvik Biology Oy, Toxicology, Turku, Finland, [2]Karolinska Institutet, The Institute of Environmental Medicine, Stockholm, Sweden

Introduction: Nanomaterials (NMs) represent a key enabling technology that would be helped by safety-by-design evaluations. Standardized high-throughput screening (HTS) for toxicity effects would fit under such a strategy from enabling evaluations of novel ENMs to be generated. We applied a tiered protocol for a 384-well HTS-based safety analysis of NMs.
Materials and methods: The BEAS-2B human lung epithelial cell line was subjected to Promega's CellTiter-Glo assay and Hoechst staining for analysis of cellular ATP content and cell number changes. Quantification of cell surface areas and apoptosis by microscopic imaging under time lapse allowed for simultaneous screening for morphological toxicity. Furthermore, high content imaging / protein lysate microarray readouts enabled antibody-based detection of specific DNA damage endpoints.
Results: Following thorough assay standardizations, established reference NMs, including oxide forms of metals, and an NM library from the NANOSOLUTIONS project caused dose-dependent toxicity over a wide range of concentrations, indicating manifold differences in potency among different NMs. Variably assessed, the dispersion protocol, storage time of the dispersions, cell density, and culture conditions were found to potentially influence the results.
Conclusion: Overall, assessment of an array of endpoints, concentrations and exposure times efficiently demonstrated influences of selected NM surface modifications. We conclude that this kind of tiered HTS-mediated toxicity evaluation approach gives access to first results within days and enables deeper and iterated evaluation of toxicity mechanism within weeks. The results argue for the value of a proactive HTS technology-mediated safety evaluation of NMs under a safe-by-design concept.

6

Cyto- and Immunotoxicity Analysis of a Panel of Nineteen Representative Nanomaterials in a Human Monocyte-Macrophage Cell Model: Results from FP7-NANOREG

Bhattacharya K., Kilic G., Fadeel B.
Karolinska Institutet, Division of Molecular Toxicology, Institute of Environmental Medicine, Stockholm, Sweden

Engineered nanomaterials are being produced for a multitude of different applications. However, like other chemicals it is important to perform safety assessment of nanomaterials during their life cycle from production, application to final disposal. Here, we performed immunotoxicity screening of 19 representative nanomaterials procured from the Joint Research Centre (JRC) of the European Commission in the frame of the FP7-NANOREG project. For the study, we tested cytotoxicity and inflammatory responses of the nanomaterials using phorbol 12-myristate 13-acetate (PMA) differentiated human monocyte cells (THP.1) as a model. Based on IC-50 values obtained at 24 and 48 h using the Alamar blue® assay, the MWCNTs, ZnO, Ag and SiO_2 nanomaterials were found to be most cytotoxic. All other nanomaterials were found to be non-cytotoxic at the time-points and concentrations tested (up to 100 µg/mL). Profiling of cytokines, chemokines and growth factors using the BioRad Human 27 cyto- and chemokine bio-plex (LUMINEX) assay indicated that the TiO_2, SiO_2, $BaSO_4$, and CeO_2 nanoparticles, as well as the nanocelluloses induced potent inflammatory responses in THP.1 cells at sub-toxic doses (10 and 25 µg/mL) following 24 h. However, the nanocelluloses were found to contain significant amounts of endotoxin, based on the LAL assay. We conclude that certain nanoparticles are cytotoxic for macrophages and that cytokine responses may be seen at non-cytotoxic doses.

7

Innovative High Throughput Modular Screening Platform for Toxicants/Nanomaterials

Mohamadi S. (.[1], Omiduro F.[2], Bryson R.[2], Hondow N.[2], Milne S.[2], Beales P.[1], Kapur N.[2], Nelson A.[1]
[1]University of Leeds, Chemistry, Leeds, United Kingdom, [2]University of Leeds, Engineering, Leeds, United Kingdom

Introduction: In recent years there has been an increase in production of nanomaterials with a variety of applications worldwide.Hence, there is a need for assessment of their possible toxicity.An innovative high throughput screening platform is introduced here,being developed into a multimodular screening system.It will incorporate selective in-built targets that mimic tissues and organs all integrated using a microfluidic network which is the heart of a new H2020 funded project HISENTS.
Materials and Methods: A biomembrane sensor is incorporated in a wafer-based platform placed in a flow cell.The electrode in the flow cell is connected to a potentiostat where capacitance current vs potential profiles are monitored using rapid cyclic voltammetry.The microfluidics allow an array of particle dispersions into the flow cell by means of integrated solenoid valves.The structures are characterised electrochemically based on modifications in structure of the lipid layer and thus its capacitance.
Results: The platform is calibrated with a series of characterised toxicants.The results show distinct patterns of behaviour dependent on the structure and shape.Interaction is indirectly related to compound hydrophobicity. The screening fingerprint of two compounds, toluene and cresol illustrates clearly the effect of =OH group in cresol enhances compound interaction.Results of gold nanoparticle dispersions screening will also be presented demonstrating nanotoxicity dependence on size and shape.
Conclusion: This technology will act as a complimentary screen to existing non-animal tests as a first stage toxicity screen of compounds and nanomaterials,filtering out those not required for further analysis.This novel approach is efficient and robust, requiring ten minutes maximum screening time for each species.

8

Lung-on-a-Chip: A Promising Model for Nanomaterials Assessment

Crespo M., Valsami-Jones É., Lynch I.
University of Birmingham, School of Geography, Earth and Environmental Sciences, Birmingham, United Kingdom

Background: As novel alternative to current in vivo and in vitro studies, organ-on-a-chip, a 3D cell culture capable of reproducing the specific inner conditions of selected organs, is presented. This novel system is based on a set of microfluidic channels able to mimic the cellular structures, and control the fluid flows and characteristic movements that define each organ, enabling the replication of equal conditions on several devices and the implementation of parallel studies. The use of transparent biocompatible materials, and the small and portable character of this device make possible its adaptation to current microscopy-based assays.
Here, we present the design and implementation of a lung-on-a-chip device, currently being assessed for its use in screening silver nanomaterials fate and cellular impacts.
Material and Methods: To compare lung-on-a-chip and submerged 2D in vitro studies, 20nm AgNPs and AuNPs were selected as test and control nanomaterials, respectively. The uptake and effects of the different nanomaterials on A549 lung cells were studied by confocal microscopy, evaluating ROS formation, protein corona evolution and lysosomal dysfunction. Techniques including DLS, UV-vis, ICP-MS and TEM were used to evaluate the nanomaterial modifications under cellular conditions.
Results: The time dependent release of silver ions in cell media was found to play an important role in the toxic effects of AgNPs on A549 cells, although delayed in the lung-on-a-chip exposure due to reduced liquid volumes.
Conclusions: Although lung-on-a-chip represents a promising tool, its complex fabrication and implementation require further studies to make it an accessible and regulatory acceptable alternative.

9

Microchip-Based Screening Tools for Enhanced Efficiency in Nanomaterial Hazard Profiling

Kohl Y.[1], *Knoll T.*[2]
[1]Fraunhofer Institute for Biomedical Engineering IBMT, Medical Biotechnology, Sulzbach, Germany, [2]Fraunhofer Institute for Biomedical Engineering IBMT, Biomedical Microsystems, St. Ingbert, Germany

Background: New screening technologies are desirable for verifying the nanotoxicity of nanoscale materials non-invasively, in real-time and with increasing complexity of biological organisation. The NanoSafetyCluster project HISENTS (High level Integrated SEnsor for NanoToxicity Screening, project start 1st April 2016) addresses this aspect. HISENTS combines multimodular screening with the PBPK model (physiologically based pharmacokinetic model). This novel approach uses a smart multi-modular screening technology for nanosafety assessment, which is needed in fields of nanotoxicology, pharmacological screenings or REACH.
Material and Methods: A miniaturized incubator microscope (MIM) with an integrated miniaturized cell culture chamber (MCC) was developed. The MIM comprises a CMOS camera and optical components for analyzing cells cultured in the MCC. The MCC comprises a microcavities with a transparent silicon nitride (Si_3N_4) membrane. The biocompatibility of the MCC was determined by viability and morphology analysis of different human cells (e.g. stem cells, neurons, lung cells) after cultivation and differentiation in the MCC.
Results: All investigated human cell models were cultured on the Si_3N_4 membrane without pretreatment of its surface. All cell lines proliferated and differentiated in the MCCs and maintained their characteristic morphologies and metabolism. Cells were cultivated over 14 days in the MIM without changes in cell characteristics. Fluorescence analysis and detection of nanoparticle-cell interaction on single cell level were successfully realized.
Conclusions: We present a pocket-sized MIM with an integrated MCC for continuous cell culturing, monitoring and investigating time-/concentration-dependent nanoparticle-induced cellular effects. The new system is therefore suitable for risk assessment in the fields of nanotoxicology or REACH.

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Flows of Engineered Nanomaterials Through Waste Treatment And Recycling

Adam V., Sultan F., Nowack B.
Empa - Federal Laboratories for Materials Science and Technology, Technology and Society Lab, St. Gallen, Switzerland

Background: Nowadays, engineered nanomaterials (ENMs) are part of our daily life, as they are present in a growing number of consumer products. Yet, their potential impacts on human and environmental health is poorly understood. More specifically, the releases of ENMs in the environment undergo large uncertainties, although they constitute the first step of environmental impact modelling.
Material and methods: This work relies on material flow analysis (MFA) to assess the flows of ENMs from their production, manufacture and consumption towards waste treatment and recycling. The national scale is used, to produce results for each European country based on the available statistics for production and waste treatment. The behavior of the ENMs during the recycling processes is also studied, in order to assess their potential elimination, release to the environment or way back to production, manufacture and consumption.
Results: This method enables the dynamic modelling of the amounts and forms of ENMs entering and exiting the recycling processes across Europe. The work focuses on the ENM nano-TiO_2, ZnO, Ag, SiO_2 and CNT. Results are presented for complete mass flows from all products using the respective ENM into waste and recycling.
Conclusions: This work constitutes the first quantitative assessment of flows of ENM to recycling in the EU. It forms an important piece in the discussion on the potential of ENM to contaminate waste flows.

11

Scaling up Nanodescriptors for Metal Oxide Nanoparticles: ZnO Case Study

Escorihuela Marti L.[1], Martorell Masip B.[2], Fernandez Sabater A.[1], Rallo Moya R.[3]

[1]Universitat Rovira i Virgili, Chemical Engineering, Tarragona, Spain, [2]Deregallera Ltd, Material Science, Caerphilly, United Kingdom, [3]Universitat Rovira i Virgili, Mathematics and Computer Sciences Department, Tarragona, Spain

Background: As widely stated in literature, physico-chemical properties of metal oxide nanoparticles (MeO NPs) are size dependent. For instance, surface free energy and stress are inversely proportional to size. Relative to bulk materials, NPs below 5 nm in diameter are extremely changing in their properties due to the high ratio of atoms on the surface. In contrast, NPs in the range of 15 to 90 nm have properties which are very similar to the bulk. Understanding the relationships between size and NP properties is fundamental to establish safe-by-desing strategies.

Materials and methods: Using the Density Functional Theory (DFT) at the generalized-gradient approximation (GGAs and GGAs+U) levels, the electronic and atomic structures for small (2 nm) ZnO NPs were computed using Quantum Espresso. Formation energies and band gaps of different NP surfaces, nanotubes and spherical NPs were evaluated.

Results: Simulation results show the difference between ZnO at bulk, which is a semiconductor (band gap of 3.2 eV at our level of theory), relative to ZnO surface structures and nanotubes which become more conductor (band gap < 1.8 eV). Taking as descriptors formation energies and band gaps of faces, nanotubes and NPs, we adjusted non-linear models capable of extrapolating descriptor values for larger nanostructures with remarkable accuracy (error < 0.1%).

Conclusions: Electronic descriptors for small structures were scaled up and, together with experimental toxicity data, will serve to develop structure-property (QSPR) and structure-activity (QSAR) relationships for larger NPs that ultimately will contribute to the computational design of NPs optimized for specific applications.

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INSIdE Nano: A Novel Computational Tool for the Contextualization of Engineered Nanomaterials (ENM) Mode of Action

Serra A.[1], Letunic I.[2], Fortino V.[3], Fratello M.[4], Kinaret P.[3], Napolitano F.[5], Ilves M.[6], Fadeel B.[7], Tagliaferri R.[1], Greco D.[3]

[1]University of Salerno, Disa-Mis, Fisciano, Italy, [2]Biobyte Solutions GmbH, R&D, Heidelberg, Germany, [3]University of Helsinki, Institute of Biotechnology, Helsinki, Finland, [4]Second University of Napoli, Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences, Napoli, Italy, [5]Telethon Institute of Genetics and Medicine, Systems and Synthetic Biology Lab, Napoli, Italy, [6]Finnish Institute of Occupational Health, Systems Toxicology, Helsinki, Finland, [7]Karolinska Institutet, Environmental Medicine, Stockholm, Sweden

In the recent years, omics technologies have been increasingly used to thoroughly characterize the engineered nanomaterials (ENM) molecular mode of action. It is possible to contextualize the molecular effects of different types of perturbations by comparing their patterns of alterations. While this approach has been successfully used for drug repositioning, to date a comprehensive contextualization of the ENM mode of action is still missing. We developed INSIdE nano (Integrated Network of Systems Biology Effects of nanomaterials), a novel computational framework for omics data integration able to highlight significant similarities between ENM, drugs, chemicals, and diseases, depending on their effects on the transcriptome. Based on the expression signature, associated to each phenotype, the strength of similarity between each pair of perturbations was evaluated and used to build a large network of phenotypes. In order to ensure the usability of INSIdE nano, we developed a robust and scalable computational infrastructure to scan this large phenotypic network and we built a web-based effective graphic user interface. Our evaluation of INSIdE nano confirmed that it highlights known disease-drug and disease-chemical connections. Moreover, disease similarity is in agreement with the information based on their clinical features, as well as drugs and chemicals, mirroring their resemblance based on the chemical structure. Altogether, our results suggest that INSIdE nano can also be successfully used to contextualize the molecular effects of ENM and infer their connections to other better studied phenotypes, speeding up their safety assessment as well as opening new perspectives concerning their usefulness in biomedicine.

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Applying "Big Data" for Handling Nanomaterials Read Across and Adverse Outcome Studies

Nymark P.[1], Rieswijk L.[2], Ehrhart F.[2], Jeliazkova N.[3], Tsiliki G.[4], Hongisto V.[5], Kohonen P.[1,5], Sarimveis H.[4], Evelo C.[2], Grafström R.[1,5], Willighagen E.[2]

[1]Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden, [2]Maastricht University, Department of Bioinformatics, Maastricht, Netherlands, [3]Ideaconsult, Ltd, Sofia, Bulgaria, [4]National Technical University of Athens, School of Chemical Engineering, Athens, Greece, [5]Misvik Biology Oy, Department of Toxicology, Turku, Finland

Emerging acceptance of the 21st century predictive toxicology paradigm couples currently with increased generation of engineered nanomaterials (ENMs)-related omics data. Read across between NMs and adverse outcome exploration within ongoing European Nanosafety Cluster projects are potentially aided by such efforts. WikiPathways provides an open collaborative platform for capturing and disseminating a full diversity of mechanisms useful for toxicity data analysis and visualization. An ENM portal created by the WikiPathways team enables community-based annotation of toxicity pathways and adverse outcomes (http://www.wikipathways.org/index.php/Portal:Nanomaterials). Risk assessors have shown caution towards" noisy" biological pathway repertoires, but focused subsets of relevant curated field-specific pathways may allow for deepened risk analysis. We report a case study for capturing existing knowledge for a toxicity pathway relevant to nanosafety. Information and omics data related to pulmonary fibrosis and high aspect ratio ENMs were extracted and mined from literature and databases (e.g. the Comparative Toxicogenomics Database). The gathered data were used to build an employable WikiPathway for this adverse outcome, representing a serious chronic respiratory condition induced by a wide variety of environmental and occupational exposures such as asbestos and silica dust. We conclude from this work that the WikiPathway templates are highly useful for computational analysis of large-scale omics data, serving diversely for: i) gene set enrichment analysis, ii) pathway enrichment analysis among differentially expressed genes, iii) integration into adverse outcome pathway-based testing strategies, iv) application as descriptors in (Q)SAR approaches, and finally, iv) for grouping and read across among NMs, coupled to identification of specific pathway-activating NMs.

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Environmental Concentrations of Nanomaterials for Six Relevant Nanoapplication Case Studies

Caballero-Guzman A., Nowack B.

EMPA, Technology and Society Laboratory, St. Gallen, Switzerland

Introduction: We model the probabilistic flows of six relevant nanoapplications into the environment: (i) Copper Oxide in wood coating, (ii) CNT in electrostatic car parts, (iii) Silica Dioxide in pancake mixtures, (iv) Titanium Dioxide used in an air filter system and (v and vi) DPP (organic pigment) and Iron Oxide used as polymer nanocomposite in car parts.
Methods: We applied the method developed by Gottschalk et al. (2010), implementing a bottom-up approach, which assess the potential market penetration of each nanoapplication into the European market. We then compared the results of the case study flows with the overall flows for each nanomaterial.
Results: The results show that in most cases the particular flows are lower than the general overall flows (less than 1%), except for Silica Dioxide and Copper Oxide, where the concentrations would increase between 6 or 50 times in Sediments and Soils respectively. The results are strongly dependent on the market penetration, which were assumed to be between 1 and 10%.
Conclusions: The preliminary results suggest an increase in the concentrations of ENM in the aforementioned compartments. In both case studies the direct release to the environment during their use phase is the largest among them. It is important to take into account that no transformations were considered in this model. Because reactive materials such as CuO will undergo transformations (e.g. dissolution), the final concentrations of the nanomaterials in the environmental compartments are expected to be lower that the resulting from this modeling approach.

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Physical and Chemical Phosphate Transformations of Zirconium Doped Ceria Nanoparticles - A Better Understanding of Engineered Nanomaterial Possible Environmental Behaviour

Briffa S.M., Lynch I., Valsami-Jones E.
University of Birmingham, School of Geography, Earth and Environmental Sciences, Birmingham, United Kingdom

Introduction: During their life-cycle many engineered nanomaterials (ENMs) undergo significant transformations that may modify their toxicity, behaviour and fate in the environment. Assessing the environmental and human health implications of ENMs requires an understanding of the potential exposure routes. This work aims to study the potential environmental transformation of commercial ceria (CeO_2) and zirconium doped ceria nanoparticles, extensively used in catalytic converters, to cerium phosphate (monazite structure).
Materials and methods: Commercial ceria and zirconium doped ceria samples were transformed by subjection to phosphatisation using 1 and 5 mM pH adjusted (c. 5.5) solutions made up of, KH_2PO_4, citric acid and ascorbic acid to simulate plant root-excreted acids. Samples were analysed before addition, on immediate addition, after 7 days and 21 days. Characterisation was carried out by DLS - size and zeta potential, UV/VIS, TEM, FT-IR, EDX and XRD so as to study the transformations occurring.
Results: Ceria and ceria-rich samples underwent transformation to larger particles growing into characteristic “sea urchin”/needle-like structures. Furthermore, these samples underwent compositional transformation to phosphate-bearing phases. Transformations were dependent on time, ceria and phosphate concentration, phosphate to ceria ratio and cerium to zirconium ratio. The zirconium oxide particles showed no transformation to phosphate.
Conclusion: Exposure to the phosphate solutions resulted in chemical and physical changes in all ceria containing samples. The zirconium presence within the doped samples did not inhibit these transformations, although pure zirconium oxide showed no transformation. The next stage of the research involves studying the transformations in more realistic environmental and biological media.

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Simulated Outdoor Use of Polypropylene Nanocomposites Containing TiO_2 Nanoparticles

Muñoz Gómez J.L.[1], Vilchez A.[1], Citterio C.[2], Vázquez-Campos S.[1]
[1]LEITAT Technological Center, Human & Envirionmental Health & Safety, Barcelona, Spain, [2]LATI Industria Termoplastici S.p.A., Product Regulatory Affairs, Vedano Olona, Italy

Background: Over the last decades has been a growing interest in engineering nanomaterials (ENM) for their potential application in different areas. The incorporation of ENM in commercial products, such as plastics, paints or textiles, has increased exponentially and they are currently present in some daily use products. However, this quick development has raised awareness to possible human and environmental risk from ENM-containing products. Such risk comprises evaluation of hazard and ENM exposure assessment. The present work aims at evaluating ENM environmental release from polypropylene nanocomposites containing embedded TiO_2 nanoparticles, under simulated outdoor conditions.
Materials and methods: Polypropylene nanocomposites containing different amounts of TiO_2 nanoparticles were studied and compared with the plain polymer with and without UV stabilizers used in the commercial formulation of the product. For each of the nine samples evaluated, 15 dogbones (standardized polymer specimens) were placed in a climatic chamber under UV irradiation and rain cycles for 500, 750 and 1000 hours. Finally, they were analysed by different techniques as TEM, SEM or FTIR (especially surface changes). Moreover, the runoff waters were collected during the rain cycles at different times and the released material analysed.
Results: Preliminary results reveal that samples without TiO_2 show the least difference in mass, before and after ageing. Additionally, the mass loss increases when the amount of TiO_2 increases.
Conclusions: The photo degradation of polypropylene increases with the concentration of TiO_2 and time exposure. TiO_2 concentration on the polymer surface rises with the exposure time.

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Strategies to Detect and to Characterize Carbon Nanotubes in the Workplace Air

Simonow B., Meyer-Plath A., Dziurowitz N., Plitzko S.
Federal Institute for Occupational Satefy and Health, Unit 4.5 'Particulate Hazardous Substances, Innovative Materials', Berlin, Germany

The fibrous morphology and the biopersistence of carbon nanotubes (CNT) and carbon nanofibers (CNF) have raised concerns about their potential lung carcinogenicity. In inhalation exposure studies with CNT/CNF, asbestos-like response reactions were partly observed. To correlate the toxicological findings to realistic human expose, it is mandatory to assess and to characterize released CNT/CNF particles in the workplace air.
Within the EU-project “NanoIndEx”, field studies in facilities handling CNT materials were performed. Direct-reading instruments and samplers were used to monitor the exposure during the handling of dry CNT materials.
By subsequent analysis of the samplers with scanning electron microscopy (SEM), we were able to detect and characterize released CNT particles at all studied sites. Interestingly, most of the used direct-reading instruments did not detect the exposure during CNT handling process, indicating that the number of released CNT was lower than the instruments lower detection limits.
The results raise the question, if direct-reading instruments, which are usually used to assess the workplace exposure towards nanoparticles, are also suitable for fibrous nanomaterials like CNT/CNF. Aerosol of fibrous (nano)materials need to be morphological characterize, as not only the number but also the particles shape is an important parameter for risk assessment. A proposal is made, how a measurement strategy for nanofiber aerosol based on SEM analysis of samplers could look like in the future.

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Exposure Assessment of a Novel Anti-Fouling Nanomaterial

Oliveira T.[1], Martins R.[2], Avelelas F.[1], Maia F.[1], Malheiro E.[1], Soares A.M.V.M.[2], Loureiro S.[2], Tedim J.[3]
[1]Smallmatek - Small Materials and Technologies, Lda, Ecotoxicology and Environmental Monitoring, Aveiro, Portugal, [2]University of Aveiro, Biology and CESAM, Aveiro, Portugal, [3]University of Aveiro, CICECO-Aveiro Institute of Materials and Department of Materials and Ceramic Engineering, Aveiro, Portugal

Background: Biofouling is a worldwide problem that requires equilibrate solutions between efficacy and toxicity. The FP7 BYEFOULING project is seeking for low-toxic cost-efficient environment-friendly antifouling materials, including for instance the encapsulation/immobilization of commercially available biocides in order to achieve control over the leaching rate. Thus, the present study was focused in the toxicity and anti-fouling efficacy of the biocide zinc pyrithione (ZnPT), the unloaded nanomaterial (LDH) and the loaded nanoclay (LDH-ZnPT) in different species, towards a future implementation of the REACH risk assessment.
Material and methods: Non-target (microalgae *Tetraselmis chuii*) and target organisms (diatom *Phaeodactylum tricornutum* and mussel *Mytilus edulis*) were exposed to ZnPT, LDH and LDH-ZnPT, from 10 µg/L to 100 mg/L total weight, during 96 h. A second exposure test with sub-lethal concentrations (lower than 100 µg/L) was carried out to assess biochemical changes caused by the tested compounds in mussels.
Results: LDH was no/low toxic for tested species. LDH-ZnPT was 10 to 100 times less toxic for both unicellular species (IC_{50}=1,970 and 3,870 µg/L for target and non-target species, respectively) than ZnPT (IC_{50}=10 and 300 µg/L for target and non-target species, respectively). In mussels, LDH-ZnPT caused higher lethality than ZnPT (LC_{50}=123 and 211 µg/L, respectively). Oxidative stress, detoxification and neurotransmission markers were not responsive, however different antioxidant patterns were found with free ZnPT and LDH-ZnPT exposures.
Conclusions: The immobilisation of ZnPT into nanoclays proved to be a promising efficient anti-fouling strategy against macrofoulers with low toxicity for phytoplankton. Nanoclays can be regarded as truly eco-friendly nanomaterials.

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Endotoxin-Free Graphene Oxide Triggers Inflammasome Activation in Primary Human Monocyte-Derived Macrophages

Mukherjee S.P.[1], *Bhattacharya K.*[1], *Lozano N.*[2], *Kostarelos K.*[2], *Fadeel B.*[1]

[1]Karolinska Institutet, Nanosafety & Nanomedicine Laboratory, Division of Molecular Toxicology, Institute of Environmental Medicine, Stockholm, Sweden, [2]University of Manchester, Nanomedicine Laboratory, School of Medicine & National Graphene Institute, Manchester, United Kingdom

Biocompatibility of graphene oxide (GO), and in particular its interactions with the immune system, should be carefully controlled for successful biomedical applications. Recent studies have suggested that GO may elicit pro-inflammatory effects. Here, we asked whether GO triggers inflammasome activation in macrophages and whether this is size-dependent. Biomaterials may be contaminated with bacterial endotoxin during production and handling, which may confound toxicological testing of these materials. Therefore, we first tested whether GO produced by the modified Hummers method was endotoxin-free using the conventional LAL assay. Our results showed that GO interfered with this assay. To overcome the problem, we devised a TNF-α expression test (TET) using primary human monocyte-derived macrophages (HMDM) incubated in presence or absence of the endotoxin inhibitor, polymyxin-B. This assay unequivocally detected endotoxin contamination in GO with comparable sensitivity to the LAL assay. Next, we assessed the interaction of endotoxin-free small versus large GO with HMDM and found that both GO flakes were readily internalized and no cytotoxicity was detected. Then, multi-plex cytokine arrays were employed for screening of inflammatory responses in LPS-primed or unprimed cells. GO induced caspase-dependent IL-1β expression, a hallmark of inflammasome activation, in LPS-primed macrophages. IL-1β secretion was NADPH oxidase-dependent, but independent of the lateral dimensions of GO. Using THP.1-based reporter cell lines, we could confirm a role for NLRP3, ASC, and caspase-1. These studies have shed new light on the biological effects of GO. Supported by the European Commission through the Flagship Project GRAPHENE, grant agreement no. 604391 and no. 696656.

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***In vitro* Assessment of Graphene and Graphene Oxide Biocompatibility at the Skin Level**

Pelin M.[1], Fusco L.[2], León V.[3], Vázquez E.[3], Tubaro A.[1], Prato M.[2]

[1]University of Trieste, Department of Life Science, Trieste, Italy, [2]University of Trieste, Department of Chemical and Pharmaceutical Sciences, Trieste, Italy, [3]University of Castilla-La Mancha, Department of Organic Chemistry, Ciudad Real, Spain

Background: Graphene-based nanomaterials (GBNs) represent a new generation of two-dimensional carbon-nanoplatforms with extraordinary physical and chemical properties, making them promising tools for nanoelectronics and biomedicine. However, little is known about their toxic effects in humans, so far. Although skin is the organ with the highest exposure potential to GBNs, scarce data are available on their safety at the cutaneous level.

Materials and methods: The effects of a ball-milled graphene (G) and a commercial graphene oxide (GO) were evaluated on human HaCaT skin keratinocytes by means of mitochondrial damage (WST-8 and JC1 assay), cell proliferation (sulforhodamine B assay), ROS production (NBT and DCFDA assay) and plasma-membrane damage (propidium iodide uptake and confocal analysis) after different exposure times up to 72h.

This study was supported by the European Union H2020 Program under grant agreement no. 696656-Graphene Flagship Core1

Results: G and GOs induced only negligible effects on cell proliferation, whereas, at high concentrations, significant mitochondrial dysfunction, ROS production and membrane damages occurred after 48 or 72h exposure with different potencies (G< GO). Confocal microscopy analysis revealed that these damages might be related to an interaction between G or GO with the plasma-membrane.

Conclusions: In conclusion, G and GO seem to significantly interact with plasma membranes of skin keratinocytes inducing a significant damage, associated with mitochondrial dysfunction and ROS production. However, these effects appear only at high concentrations after long exposure times suggesting an acceptable biocompatibility of these nanomaterials with skin keratinocytes, at least after exposure as long as 24 hours.

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Double-Edged Sword: Antioxidant Cerium Oxide Nanoparticles Protect Against Oxidative Insult and Inhibit Differentiation of Neural Stem Cells

Gliga A.R.[1], Edoff K.[2], Caputo F.[3,4], Källman T.[5], Karlsson H.L.[1], Ghibelli L.[3], Traversa E.[4], Ceccatelli S.[2], Fadeel B.[1]

[1]Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden, [2]Karolinska Institutet, Department of Neuroscience, Stockholm, Sweden, [3]University of Rome 'Tor Vergata', Department of Biology, Rome, Italy, [4]University of Rome 'Tor Vergata', Department of Chemical Science and Technology, Rome, Italy, [5]Uppsala University, Department of Medical Biochemistry and Microbiology, Uppsala, Sweden

Cerium oxide nanoparticles (nanoceria) have shown promising neuroprotective effects related to their antioxidant properties, both *in vitro* and *in vivo*. The aim of the present study was to investigate the interaction of nanoceria with neural stem cells, using the murine cell line C17.2. We assessed the cytoprotective effect of nanoceria *vs.* samarium (Sm) doped nanoceria with a reduced antioxidant activity following oxidative insult (DMNQ, dimethoxy-naphthoquinone) and the impact of these particles on neuronal differentiation. Cellular uptake was quantified by ICP-MS, intracellular localization was visualized by TEM, while cell viability following oxidative insult was monitored by automated microscopic morphological assessment (Cell-IQ assay). Finally, neuronal differentiation was determined by immunofluorescence (β3-tubulin) and next-generation sequencing (RNA-Seq) was employed to determine genome wide changes at the transcriptional level. Both nanoparticles were non-cytotoxic and were internalized by proliferating and differentiating C17.2 cells. Nanoceria, but not Sm-doped nanoceria, elicited a transient cytoprotective effect following DMNQ exposure. Next, we assessed the effects on neuronal differentiation, which was induced by serum deprivation and N2 supplement addition. Nanoceria, but not the Sm-doped particles, reduced β3-tubulin expression, an early marker of neuronal differentiation. Additionally, network and pathway analysis of the RNA-Seq data using the Ingenuity Pathway Analysis tool revealed that nanoceria significantly reduced expression of genes involved in neuronal development, neuronal differentiation as well as cytoskeletal organization. Taken together, the present *in vitro* study provides evidence for a dual effect of nanoceria on neural stem cells, *i.e.*, promotion of neuronal survival under oxidative stress conditions and suppression of neuronal differentiation.

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Quantification of Silver Nanoparticle Uptake and its Cellular Distribution in Caco-2 Cells by Means of Spatial Resolved Element Imaging Techniques

Meyer T.[1], Lichtenstein D.[2], Lampen P.D.D.A.[2], Estrella-Lopis D.I.[1]
[1]University of Leipzig, Institute for Medical Physics and Biophysics, Leipzig, Germany, [2]Federal Institute for Risk Assessment, Department Food Safety, Berlin, Germany

Due to its antimicrobial properties, silver nanomaterials (NMs) are widely used for food and packing industries. This leads to a high oral intake. Due to the role of silver nanomaterials in food related products it is of major importance to investigate particle uptake after oral exposure. An *in vitro* experimental setup, using differentiated Caco-2, was applied to mimic this situation. Three differently surface modified silver nanomaterials were chosen to gain knowledge about cellular response, uptake behavior, internalization and intracellular distribution of the particles as a function of the surface modification on a sub toxic silver concentration.
By using label free Ion Beam Microscopy (IBM) techniques, combining µPIXE (micro-Particle-Induced-X-Ray-Emission) and µRBS (micro-Rutherford-Backscattering-Spectrometry) with a spatial resolution of 1 µm; it is possible to gain 3-dimensional information about localization and effective intracellular concentration of the applied NMs. Furthermore, one can calculate the elemental concentration of cellular matrix and trace elements in the range of parts per million.
It was shown that the uptake and aggregation behavior strongly depends on the surface modification of the silver particles. The applied NMs had a significant impact on the concentration of metabolically relevant trace elements. Results from IBM compared to Atomic Absorbance Spectroscopy and TEM exhibits good accordance between the methods.
IBM was proven to be a successful tool for quantification of uptake and 3D visualization of NMs down to the single cell level. Genuine cellular concentration could be considered as new toxicological relevant endpoint which is the central link between exposure and toxicity.

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***In situ* Characterization of Protein Corona Nanoparticles by Fluorescence Correlation Spectroscopy**

Di Silvio D.[1], Silvestri A.[2,3], Polito L.[2], Moya S.[1]
[1]Centro de Investigación Cooperativa en Biomateriales CIC biomaGUNE, Soft Matter Nanotechnology, San Sebastian, Spain, [2]CNR - ISTM, Nanotechnology Lab, Milan, Italy, [3]University of Milan, Department of Chemistry, Milan, Italy

Nowadays, given the wide range of applications of engineered nanomaterials (ENMs) it is extremely important to relate their physical-chemical properties to the biological fate in terms of cellular uptake and potential cytotoxicity to improve targeting activity in nanomedicine applications and for safety issues related to the general use of nanomaterials. One of the most interesting aspects of the ENMs is their behavior with the biological environment. In fact, physical-chemical features of ENMs (core, surface coating, shape and size) affect the formation of a biological corona around them as soon as they are exposed to the biological environment and the biological corona, formed by proteins, carbohydrates, and/or lipids, constitutes the ultimate NPs interface with the surroundings. The aim of this work is to study the protein corona for several ENMs differing in core, size and surface coatings using Fluorescence Correlation Spectroscopy (FCS). In particular, the effect of polyethylene glycol moieties and glucose on the coatings will be evaluated independently and together. FCS allows studying *in situ* fluorescently labeled species such as proteins, NPs, molecules through their diffusion within a confocal volume. The diffusion coefficient is related to the hydrodynamic size of the species. Moreover, the diffusion of NPs gives indication about their localization in cell compartments in the way that the uptake of NPs can be followed with and without corona. Results will be correlated to *in vitro* cells uptake studies by Flow Cytometry.

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Interactions of Dendritic Polyglycerols with Serum Proteins Affects Cellular Uptake

Bewersdorff T.[1], *Vonnemann J.*[2], *Kanik A.*[1], *Haag R.*[2], *Haase A.*[1]
[1]The Federal Institute for Risk Assessment (BfR), Department 7: Chemicals and Product Safety, Berlin, Germany, [2]Freie Universität Berlin, Institute of Chemistry, Berlin, Germany

Background: Dendritic polyglycerols (dPG) structures are interesting carrier systems in nanomedicine. They are biocompatible and can be synthesized with different functionalities. However, often binding of plasma proteins hampers the medical application due to enhanced cellular uptake and clearance by the reticuloendothelial system. Therefore, it is essential to understand the bio-nano-interface of those NPs.
Material and Methods: Two sulphated (dPGS) of different sizes and one non-sulphated (dPGOH) dPG nanocarriers were used in our study. We identified the serum protein corona via 2D gel electrophoresis in combination with MALDI-TOF/TOF and studied the influence of the corona on cellular uptake via flow cytometry. Cell membrane proteins of THP-1 cells were purified using affinity chromatography after biotinylation and used for pull-down-experiments with protein corona coated dPGS. We then identified interacting membrane proteins via nanoHPLC-MALDI-TOF/TOF.
Results: We could identify the protein coronas for all dendritic polyglycerols. Furthermore, we analyzed cellular uptake and found that dPGS was taken up much more efficiently compared to non-sulphated dPGOH. The presence or absence of a protein corona had a significant impact on cellular uptake in vitro. Finally, we could identify 10 cell membrane proteins, which might be involved in cellular uptake via the pull down approach. Currently we investigate the mechanisms of cellular uptake.
Conclusions: We found that the surface coating and charge had a strong influence on the corona composition while size was not as important. The protein corona strongly affected cellular uptake. Our pulldown approach allowed to identify membrane proteins which are possibly involved in cellular uptake.

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Release of Nanosized Titanium Dioxide (TiO_2) in Photocatalytic Commercialized Paint Products

Bartolomei V., *Boutry D.*
CEA Grenoble, DTNM/SEN/LR2N, Grenoble, France

Background: NanoParticles (NPs) are more present than ever in commercialized products (building, cosmetic, food …). This study focuses on paint products in which micro or nano titanium dioxide (TiO_2) can be added depending of the desired property. Microsize TiO_2 particles are usually used to provide white color to paints whereas nanosize TiO_2 brings a photocatalytic effect to purify environment.
Material and methods: Preliminary study was performed on two paints containing micro and nano TiO_2. Those paints were investigated before and after ageing process alternating UV B irradiation and water spray. XPS analysis was used to characterize the near surface degradation of samples and abrasion was done to simulate mechanical ageing. The photocatalytic activity was also measured by the reduction of a Volatile Organic Compound (VOC), i.e. Xylene, with a flow tube reactor coupled to a PTR MS.
Results: XPS observations revealed slight changes between both micro paints (before and after ageing), contrary to the paint containing NPs which showed a complete matrix degradation after ageing. An important Xylene degradation was observed only for the aged paint containing NPs due to the binder disappearance. That paint also showed no more emission of VOCs, confirming the organic binder consumption. After climatic ageing, a thin surface layer of TiO_2 NPs was observed leading to a large NPs aerosol formation during abrasion process.
Conclusions: As polymer matrix is degraded too rapidly by photocatalytic process, new types of NPs will be synthesized in a Safer by Design approach to obtain a more durable product.

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Copper Toxicity in Collembola: A Comparison between Nano and Non-Nano Agrochemicals

Neves J.[1], Cardoso D.N.[1], Malheiro C.[1], Soares A.M.V.M.[1], Wrona F.[2,3], Loureiro S.[1]
[1]University of Aveiro, Department of Biology & CESAM, Aveiro, Portugal, [2]University of Calgary, Department of Biological Sciences, Calgary, Canada, [3]Alberta Environment and Parks, Environmental Monitoring and Science Division, Edmonton, Canada

Nanotechnology applications in agriculture have been growing over the past decade especially in the form of nanoagrochemicals. These products are considered as one of the main emission sources of nanomaterials in terrestrial ecosystems and there are concerns about their behaviour in soils. The objective of this work was to compare the toxicity and bioavailability in soil porewater of a copper nanopesticide with other non-nano copper pesticides and their correspondent active ingredient using the model organism *Folsomia candida*. Standardized reproduction bioassays were performed with $CuSO_4$, $Cu_2Cl(OH)_3$, and $Cu(OH)_2$ agrochemicals and respective active ingredients. Their toxicity and behaviour were tested in two soils: Lufa 2.1 and 2.2 with two times for spiking equilibrium (0 and 48 hours). The results showed that the active ingredient alone is more toxic than the correspondent commercial products, being the $Cu(OH)_2$ the one with the lowest EC_{50s} in Lufa 2.1, 48h of equilibrium. The same pattern was also observed with all the analogue pesticides tested, having the nanopesticide presented one of the most toxic pattern. Comparing the two soils it is possible to see an increase in toxicity in Lufa 2.1 probably related to the soil itself (lower performance showed by collembolans) but also to the lower pH and lower organic matter content that promotes the bioavailability of metals in soils by increasing solubility. This study helps to better understand how the unique behaviour of nanomaterials affect their toxicity and will allow improving the hazard assessment and identify the potential pathways for environmental effects related to nanoagrochemicals.

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Internalization and Toxicological Mechanisms of Cerium Oxide Nanoparticles in an Environmentally Relevant Organism

Pulido-Reyes G.[1], Briffa S.[2], Yudina T.[3], Leganes F.[1], Puntes V.[3], Valsami-Jones E.[2], Rosal R.[4], Fernandez-Piñas F.[1]
[1]Universidad Autonoma de Madrid, Biology, Madrid, Spain, [2]University of Birmingham, School of Geography, Birmingham, United Kingdom, [3]Catalan Institute of Nanoscience and Nanotechnology, -, Barcelona, Spain, [4]University of Alcalá de Henares, Ingenieria Quimica, Madrid, Spain

Background: Cerium oxide nanoparticles (CNPs) are increasingly used in industrial applications and may be released to the aquatic environment. The toxicological mechanism of these nanoparticles is still poorly understood and little is known about the internalization process of CNPs in algae due to the thick cell wall. So, we have used an uncoated and different polyvinylpyrrolidone-coated CNPs with the aim of identifying their internalization and toxicological mechanisms.
Material and methods: We have synthesized an uncoated and three PVP-coated-nanoparticles with different PVP-chain lengths (10, 40 and 360kDa). Growth inhibition experiments using *Chlamydomonas reinhardtii* were performed as described in OECD TG-201. Several cytotoxicity biomarkers (cell membrane integrity, oxidative stress…) were analyzed by flow cytometry. Different endocytic inhibitors were used to study which endocytic mechanism, if any, may be involved.
Results: The nanoparticle without coating was more toxic at low concentrations than PVP-coated CNPs. However, there was a high growth inhibition for a concentration of 50 mg/L in CNP-PVP10 and CNP-PVP40. Only CNP caused a disruption of the cell membrane integrity. Moreover, CNPs with PVP caused a remarkable accumulation of ROS in microalgae cells and affected other physiological parameters.
Conclusions: The toxicological mechanism of the uncoated nanoparticle was through damage in cytoplasmic membrane. Regarding PVP-coated-nanoparticles, the mechanisms of toxicity relied primarily on the intracellular ROS formation, however different degrees of cell damage were found depending on PVP chain length. Cerium internalization was found with all nanoparticles. Studies with endocytosis inhibitors indicated that clathrin-dependent endocytosis might be the main pathway of nanoparticle entry.

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The Effect of Ag NPs on Isopods Is Interplay of the Shape, Size and Dissolution of Particles: A FTIR Study

Novak S.[1], Romih T.[1], Drasler B.[1], Sorieul S.[2], Vaccari L.[3], Ferraris P.[3], Birarda G.[3], Zieba M.[4], Sebastian V.[4], Arruebo M.[4], Hočevar B.S.[5], Jemec A.[1], Drobne D.[1]

[1]Biotechnical Faculty, Department of Biology, Ljubljana, Slovenia, [2]Centre d'Études Nucléaires de Bordeaux-Gradignan, Service Instrumentation/AIFIRA, Bordeaux, France, [3]Elettra - Sincrotrone Trieste S.C.p.A., SISSI - Synchrotron Infrared Source for Spectroscopy and Imaging, Trieste, Italy, [4]Institute of Nanoscience of Aragon (INA), Department of Chemical and Environmental Engineering, Zaragoza, Spain, [5]Institute of Chemistry Slovenia, Analytical Chemistry Laboratory, Ljubljana, Slovenia

Isopods were fed with cubical NPs (NCs) and two sizes of spherical NPs (NSs) at very low concentrations (3 and 30 µg Ag/g of food for NCs and 0.28 and 2.8 and 0.3 and 3 µg Ag/g of food for NSs). The aim of the study was to investigate which of the properties of AgNPs - size, shape or dissolution potential - plays the predominant role in the biological effect on digestive tissue of isopods.
We used Fourier transform infrared microscopy (FTIRM) to compare the spectral patterns of digestive glands of animals exposed to different types of AgNPs, as well as to link these data to Ag accumulation and conventional toxicological parameters.
Feeding behavior, weight change and survival were not affected upon exposure to AgNPs. The accumulation of Ag ions in digestive glands was evident. FTIRM showed that the exposure to Ag NCs at both concentrations and to lower concentrations of AgNSs resulted in protein conformational changes and increase of carbohydrates. A more severe response was characteristic for spherical AgNPs at higher exposure concentrations revealing more unsaturated lipids and protein conformational changes. FTIR revealed the specific effects in the digestive tissue of the exposed animals, where exposure to AgNSs of higher concentrations caused different molecular fingerprint as AgNCs and AgNSs of lower exposure concentrations. The results have shown that size, shape and Ag NPs dissolution mutually contributed to a distinct molecular response in the tissue at subtoxic exposure concentrations.

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Translocation of CeO_2 and $BaSO_4$ Nanoparticles in Rats after 90 Days Inhalation Study Using Molecule and Element Based Imaging Techniques

Venus T.[1], Estrela-Lopis I.[1], Schwotzer D.[2], Niehof M.[2], Ernst H.[2], Creutzenberg O.[2]

[1]University of Leipzig, Institute for Medical Physics and Biophysics, Leipzig, Germany, [2]Fraunhofer Institute, Institue for Toxicoloy and Experimental Medicine, Hannover, Germany

In this study, the potential toxic effects of Nanoparticle (NP) exposure should be determined. Therefore, Wistar rats were exposed to low dosage of NPs (0,1/0,3/1/3 mg/cm^3 CeO_2 and 50mg/cm^3 $BaSO_4$) over the course of 90 days with an additional recovery phase of 90 days. The aim is to establish and validate new sensitive endpoints as early indicators for the prediction of cancerogenic/toxic effects, which will be verified in a subsequent 2-year long-term chronic inhalation study in female Wistar rats (NanoREG, BASF).
Isolated alveolar cells type II (AT II) as well as lung sections of the treated animals were analysed using Raman Microspectroscopy and Ion Beam Microscopy. Additional *in vitro* experiments in A549 cells were done using Confocal Laser Scanning (CLSM) Microscopy and Flowcytometry.
The NP uptake was quantified and the distribution patterns on subcellular levels were analysed *in vivo* and *in vitro. In vivo* studies revealed an accumulation of CeO_2 and $BaSO_4$ NPs in isolated AT II cells and the septum of the lung. CeO_2 showed a typical endocytotic distribution pattern in the cytoplasm, whereas $BaSO_4$ were found to be co-localized with the endoplasmic reticulum. Ion Beam analysis revealed also an influence of CeO_2 on the homeostasis of certain elements, like iron or zinc, which was not found after $BaSO_4$ treatment. In addition, Flow Cytometry and CLSM analysis showed a potential genotoxic effect of CeO_2.
Furthermore, clusteranalysis of biochemical changes will help evaluating these effects regarding their ability to serve as biomarkers for potential toxic and cancerogenic impacts.

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ABSTRACTS OF POSTER PRESENTATIONS

1

Can Organic Degradation Products Stabilize Metal Nanoparticles in Solution?

Pradhan S.[1], Hedberg J.[1], Blomberg E.[1], Odnevall Wallinder I.[1], Wold S.[2]
[1]KTH Royal Institute of Technology, School of Chemical Science and Engineering, Surface and Corrosion Science, Stockholm, Sweden, [2]KTH Royal Institute of Technology, School of Chemical Science and Engineering, Division of Applied Physical Chemistry, Stockholm, Sweden

Background: The stability of metal nanoparticles (MNPs) in solutions of varying chemistry needs to be studied to understand their effects on the environment and living organisms. To be able to predict where the particles will end up and in what form, the stability and dissolution are key parameters.
Material and Methods: Copper, manganese, and aluminum NPs were studied. Particles were characterized using TEM, zeta potential, Raman, pH titration and electrochemical methods to assess their physico-chemical properties. Particle stability was studied by measuring concentration of particles with time by dynamic light scattering and nanoparticle tracking analysis. Atomic absorption spectroscopy was used to measure the metal concentration in solution with time, which correlates to the metal release. The MNPs were dispersed in freshwater (OECD) with or without the addition of model organics, dihydroxy benzoic acid.
Results: The study showed that the small organic molecules slightly increased the particle stability initially. In 24 h the amount of sedimented particles was comparable to the corresponding system without any organic molecules. pH titration studies showed no strong covalent interaction between the organic molecules and MNPs. Further spectroscopic studies will be performed to investigate surface interactions.
Conclusion: The DHBA model molecule was not able to stabilize the MNPs dispersions. Dissolution was evident, most pronounced for Cu NPs followed by Mn and Al NPs. The interaction between DHBA and the Al NPs is likely electrostatic and not governed by covalent interactions due to opposite charges of the DHBA (negative) and the Al NPs (positive) at given conditions.

2

Characterisation and Cytotoxicity Assessment of the Emissions of a CdTe Quantum Dot Based Fluorescent Ink: NANOSOLUTIONS Case Study

Blázquez-Sánchez M.[1], Inge N.[2], Frijns E.[2], Van Laer J.[2], An J.[2], Pomar-Portillo V.[3], Fernández-Rosas E.[3], Vilchez-Villalba A.[3]
[1]INKOA SISTEMAS, SL, RTD Project Manager, Bizkaia, Spain, [2]VITO, Environmental Risk and Health Unit, Boeretang, Belgium, [3]Leitat Technological Center, Materials Safety Unit, Barcelona, Spain

Background: In the present work, printing process of CdTe quantum dot based fluorescent ink is evaluated by means of lab-scale realistic simulations in order to assess the impact of released materials on humans and the environment basing on characterisation and cytotoxicity measurements.
Materials and methods: Water based ink containing polyethyleneglycol-coated CdTe quantum dots has been obtained from PlasmaChem GmbH. Household printing simulations have been performed using a Canon iP7250 printer inside a hermetic test chamber with purified air. · Emissions characterization. The chamber contained Scanning Mobility Particle Sizer (SMPS) and a Condensation Particle Counter (CPC) with filters and copper grids for SEM and TEM characterisation. Cytotoxicological effect evaluation Aerosolized ink has been exposed to BEAS-2B cell line placed in a VITROCELL® module with the aim to simulate the human inhalation risk due to the ink printing process.
Results: · Emissions characterization: An increment of number of particles during printing has been detected and CdTe quantum dots have been observed by electronic microscopy (SEM and TEM with EDX) demonstrating that a release occurs. Cytotoxicological effect evaluation BEAS-2B cells presented 70% viability in the air-liquid interface after 1 hour of exposure, respect clean air atmosphere measurements.
Conclusions: It has been possible to test the human toxicological potential of ENMs released from nano-enabled products using emissions of real life cycle simulations. Moreover, it has been demonstrated that a release to the environment occurs from the printing process and that it can have an impact to the humans when inhaled.

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Detection and Characterization of Titanium Nanoparticles (TiO_2) in Food by Asymmetric Flow-Field-Flow-Fractionation - Inductive Coupled Plasma - Mass Spectrometry (A4F-ICP-MS)

Givelet L.[1], Boutry D.[1], Motellier S.[1], Jitaru P.[2], Guérin T.[2], Damlencourt J.-F.[1]
[1]CEA Grenoble, CEAGRE/SPNS/LR2N, Grenoble, France, [2]Université de Paris-Est, Anses, Laboratoire de Sécurité des Aliments, Maisons-Alfort, France

Background: Titanium dioxide is a synthetic dye for food known as E171 and used to enhance the white color. Recently, publications showed that this additive is partially present at a nanoscale size (< 100 nm). NanoParticles (NPs) size distribution can be determined by numerous analytical techniques including Asymmetric Flow-Field-Flow-fractionation coupled with Inductive Coupled Plasma-Mass Spectrometry (A4F-ICP-MS). A4F allows particle separation based on their size and z potential (electrostatic interaction) and requests optimization of several parameters to improve separation (e.g. membrane nature, flow…). The aim of this work is to optimize all these parameters for TiO_2 analysis.
Material-Methods: A Zetasizer instrument was used to measure the z potential and the hydrodynamic diameter of TiO_2 NPs in suspension in different media. An Electrokinetic Analyser SurPASS instrument was used to determine the z potential of different A4F common membranes. These measurements helped us to choose the carrier liquid and membrane to minimize particle-membrane interactions.
In parallel, several E171 samples were characterized to determine specific surface, density and structure
Results: Results showed a significant impact of the pH on z potential whether for particles or membranes. Furthermore, ionic strength, surfactant nature and concentration also had an effect on z potential. Deionized water and FL-70 seemed suitable for A4F application.
Concerning the characterization of E171, results confirmed the presence of TiO_2 NPs in anatase form.
Conclusions: This preliminary study helped us to select carrier liquid and A4F membrane to develop an A4F-ICP-MS analytical method to quantify and determine the E171 size distribution in food samples.

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Exploring Correlations Patterns on Toxicity Omics Data

Varsou D.D.[1], Tsiliki G.[1], Drakakis G.[1], Nymark P.[2], Kohonen P.[2,3], Chomenidis C.[1], Doganis P.[1], Grafström R.[2,3], Sarimveis H.[1]
[1]National Technical University of Athens, School of Chemical Engineering, Athens, Greece, [2]Karolinska Institutet, Institute of Environmental Medicine, Stockholm, Sweden, [3]Misvik Biology Oy, Department of Toxicology, Turku, Finland

Background: High-throughput experimental methods such as RNA deep sequencing transcriptomic approaches, oligonucleotide microarrays and mass spectrometry (MS) experiments, are becoming increasingly popular and their usefulness has been demonstrated on nanoparticle (NP) data (McDermott et al., 2013; Walkey et al., 2014). Recently, many studies have demonstrated that by integrating omics data with genomic knowledge to construct pre-defined features, results in higher performance in predicting clinical outcomes or profiles and higher consistency between the results of different studies (Yang et al., 2012; Balbin et al., 2013).
Material and methods: Along these lines, predictive models are considered for proteomics and genomics NP data where data are filtered based on pathway information from well-known databases, such as the signature database MSigDB, and physicochemical descriptors. For that reason we are employing gene set enrichment as well as clustering techniques and similarity read-across measures.
Results: High accuracy values (R2>=0.89) are reported for NP data depending on the filtering of the data, the number of neighbours or the distance metric considered (cosine similarity, Manhattan distance etc). Results for two use cases are presented, namely for a protein corona data set and microarray NP data.**Conclusions:** Our findings suggest that the use of constitutional genetic variation to predict toxicity response increases performance considerably. Results could be easily incorporated in QSAR models, could be used to identify next-generation biomarkers for toxicity, or to form the basis for comparative toxicogenomics modelling.

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Highly Reactive Oxygen Plasma Treatment Improve Biocompatibility of Tio_2 Surfaces with Human Osteoblasts and Osteoblast-Like Cells *in vitro*

Rugelj N.[1], Drašler B.[1], Junkar I.[2], Kulkarni M.[3], Kononenko V.[1], Iglič A.[3], Mozetič M.[2], Drobne D.[1]
[1]Biotechnical Faculty, University of Ljubljana, Department of Biology, Ljubljana, Slovenia, [2]Jožef Stefan Institute, Department of Surface Engineering and Optoelectronics, Ljubljana, Slovenia, [3]Faculty of Electrical Engineering, University of Ljubljana, Laboratory of Biophysics, Ljubljana, Slovenia

Introduction: Due to their unique mechanical and chemical properties titanium and titanium alloys are widely used in medicine as orthopaedic, dental or vascular implants. Among them titanium dioxide (TiO2) nanotubes arrays are one of the most promising candidates for implant application since several *in vitro* studies have demonstrated higher cell adhesion and proliferation when cultured on nanotubular surfaces. Another approach to improve the surface layer of biomaterial without influencing on its bulk attributes is gaseous plasma treatment. Thus, by combining the surface nanotopography features and plasma modification, a desired biological response may be achieved.
Materials and methods: In the present study, we employed gaseous plasma treatment technology to alter the oxide layer of TiO2 nanotubes and to study the influence of such modification on *in vitro* biocompatibility with primary human osteoblasts (HOB) and human osteoblast-like cell line (MG-63). Cell adhesion and morphology were estimated based on scanning electron microscopy images, whereas cell viability upon exposure to different TiO2 surfaces was assessed spectrophotometrically.
Results: The obtained results showed that oxygen plasma treated TiO2 nanotube surfaces did not enhance cell proliferation; however, adhesion and morphology of both HOB and MG-63 cells were significantly improved, especially in the case of nanotubes with 100 nm in diameter.
Conclusion: Plasma treatment induces alterations in chemical surface composition of the TiO_2 nanotube surfaces, reflecting in an improved cell adhesion on the surface. Surface finishing of orthopaedic or dental implants by oxygen plasma could therefore have an important role in medical implications of the implants.

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Impact of Surface Modifications of CuO ENMs on their Toxicity, Uptake and Localization in A549 Cells

Merker C., Estrela-Lopis I.
University of Leipzig, Institute for Medical Physics & Biophysics, Leipzig, Germany

Modifying the surface properties of engineered nanomaterials (ENMs) by attaching functionalized groups is of paramount importance to control and understand their potentially toxic impact as well as their translocation, localization and interaction with living organisms at cellular levels.
The uptake and distribution of surface modified CuO ENMs across plasma membranes was studied in human lung epithelial adenocarcinoma cells (A549) by means of space resolved micro- Proton induced X-ray emission (μPIXE) and micro-Rutherford-backscattering (μRBS). The both techniques were applied simultaneously to quantify and visualize the distribution of copper and trace elements at single cell level. Furthermore, cellular responses to ENMs were studied by means of flow cytometry. Ratio between living, apoptotic and necrotic cells (annexin V/ propidium iodide staining) as well as mitochondrial membrane potential (JC-1 staining) and the generation of reactive oxygen species (ROS) were determined.
μ-PIXE was able to detect the particulate distribution pattern of PEGylated and carboxylated ENMs as well as dissolved CuO NMs in case of pristine and CuO-NH_3^+ nanomaterials. These findings correlated well with toxicity studies of surface modified CuO ENMs, where the toxicity strongly depended on the type of surface modification. The results support an assumption that PEGylation has a high protective potential and could prevent the release of Cu ions at least over 48 hours even though uptake rate of PEGylated CuO ENMs was three times higher compared to that of unmodified particles.

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Impact of the Cross-Talk Of Enteric Human Bacterial Pathogens with Nanoparticles

Westmeier D.[1], Docter D.[1], Wessler S.[2], Knauer S.[3], Stauber R.[1]
[1]Department of Nanobiomedicine, University Medical Center of Mainz, Mainz, Germany, [2]Department of Microbiology, Paris-Lodron University of Salzburg, Salzburg, Austra, [3]Institute for Molecular Biology, Centre for Medical Biotechnology (ZMB), University Duisburg-Essen, Essen, Germany

Introduction: Enteric bacterial pathogens can cause various severe diseases. Also, scientific and medical interest in the human microbiome, defined as the sum of all microbial organisms residing inside the body, has increased dramatically. Notably, bacterial infection path overlap with the oro-gastrointestinal exposure route for nanoparticles (NPs), occurring during environmental exposure or deliberate medical applications. Hence, it is surprising that the interaction of NPs with bacteria and its (patho)biological consequences have not yet been investigated in detail. Most studies rather focussed on the anti-bacterial potential of NPs.
Material and Methods: As the physico-chemical characteristics of NPs (co)define their behaviours and (patho)biological activity in physiological systems, we studied a library of various model NPs widely varying in size, material, shape, and surface functionalisation. The interaction of NPs with different pathogenic and apathogenic enteric bacteria as well as the impact of NPs on bacteria-host cell responses was investigated by comprehensive analytical approaches, including live cell fluorescence microscopy, biochemical assays, electron microscopy, and high-throughput analysis.
Results: Here, we show that various NPs rapidly formed stable complexes with different bacteria, including pathogens such as *Helicobacter pylori* or *Listeria monocytogenes,* but showed no anti-bacterial effects. NP-coating was affected by physiological parameters including pH or temperature, and significantly affected the (patho)biological identity of both, the bacteria and the NPs. In particular, we focused on *Helicobacter pylori,* involved in the development of gastric cancer. Here, we found reduced CagA phosphorylation and IL-8 secretion when enteric AGS cells were infected with NP-coated *versus* pristine bacterial cells.**Conclusion:** We demonstrate for the first time that that the coating of enteric bacteria with NPs can impact the (patho)biological outcome and fate of exposure of the human host to both, NPs and bacterial pathogens. We expect that the identified mechanism will be of biomedical and toxicological relevance for other pathogens as well as the human microbiome in general.

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Microbial Toxicity of Cadmium Telluride Quantum Dots towards *Escherichia coli* K-12 (Guyer)

Vassallo J., Boden R., Handy R.D.
University of Plymouth, School of Biological Sciences, Plymouth, United Kingdom

Background: Quantum dots consist of semiconductor nano-sized particles, with an outer coating layer determining their stability and/or biological design requirements. Industrial-scale production of nanomaterials raises concerns about novel or unforeseen properties.
Material and Methods: The minimum inhibition concentration assay was used to screen cadmium telluride quantum dots (CdTeQD) having a carboxylate, ammonium or polyethylene glycol (PEG) coating against *Escherichia coli* K12 (Guyer). The toxicity was compared to that of bulk CdTe and cadmium chloride. Actively growing cultures (basal medium with 10mM glucose as sole carbon and energy source) were exposed to a dilution series of the test suspensions in 96-well plates (n = 6 plates/treatment). A test suspension exposure, at identified sub-lethal concentrations, followed in Erlenmeyer flasks (n = 3 flasks/treatment). The magnitude of growth inhibition was chosen as the end-point. The amount of glucose remaining after each exposure was measured.
Results: For all test suspensions, nanoparticle tracking analysis revealed natural particle aggregation and dialysis experiments showed minimal dissolution of cadmium and tellurium at pH 6.5. Complete microbial growth inhibition was recorded at 6 mg/L for the ammonium-coated, 25 mg/L for the carboxylate-coated and 100 mg/L for the PEG-coated CDTeQD. CdTe bulk and cadmium chloride were not found to be toxic to the microbe.
Conclusions: The size of the quantum dots may be an important factor that determines their toxic effect, irrespective of the physico-chemical properties of the exposure medium. On-going work is assessing glucose metabolism and the resulting carbon yields from the sub-lethal concentration exposures of nanomaterials.

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Nanoparticle Deposition On a Surface Colonised by Bacterial Polysaccharide

Dzumedzey Y.[1], Labille J. [1], Cathala B. [2], Moreau C. [2], Santaella C. [3]
[1] CEREGE, Aix-Marseille University France, [2] INRA BIA, France,[3] LEMIRE – CEA France

During last years, the increase in the production of manufactured nanomaterials and in the number of their applications in commercial products has led to an emerging concern regarding their potential impact. Nanomaterials may release into the environment some nanoscale residues such as nanoparticles (NPs) that may pose a risk depending on their exposure and hazard. The mobility and fate of NPs in the environment drive the exposure aspect. Heterogeneities in the continuity and composition of the aqueous environment, e.g. suspended particulate matter, porous media, constitute as many natural collector surfaces that will possibly interact with NPs and influence their further mobility and behavior. Thus, it is crucial to be able to estimate the affinity of the NPs for such surfaces.
Moreover, in humid environment, most of the mineral surfaces are coated with biofilms (a self-produced matrix of extracellular polymeric substance, EPS), which may act as a natural porous and organic substrate retaining or repelling the NPs. The actual effect of the EPS substrate on the NP fate depends on the NP affinity for these organic components.
The objective of this study was to study the effect of an organic EPS coating on a collector surface on the affinity and deposition of TiO_2 NPs. The collector used was SiO_2 pure or coated with a polysaccharide layer. In our approach, NP deposition was studied under favorable or unfavorable conditions. This was achieved by changing the electrostatic interactions with the collector from attractive to repulsive, through NP coatings (bare or PAA-coated), pH (< pH_{IEP}, > pH_{IEP}) and salt concentration. The affinity of the NP for the EPS and SiO_2 was studied by deposition on the surface in different conditions utilizing Atomic Force Microscopy (AFM) and Quartz Crystal Microbalance with Dissipation (QCM-D).
Our results show that the physicochemical conditions influence strongly the mode of NP deposition. Under attractive interaction, the NP deposit density increased with the ionic strength for both collector surface types. This was due to the decreasing electrostatic repulsions between neighbour nanoparticles. Higher deposition was also evidenced on the mineral collector in comparison with the EPS coated one in the same conditions. This was certainly due to the roughness of the EPS layer, which decreased the stability of the NP layer. The thickness analysis of the NP deposit on the substrate revealed that multilayer was never formed. Under repulsive electrostatic interactions between the NP and the EPS-coated collector, an unexpected NP deposition, partially reversible, was measured. This was probably due to the EPS swelling that favoured mechanical catchment of the NP.The sticking efficiency resulting from the affinity of NPs to the substrate as finally calculated from QCM-D data.

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Predictive Toxicogenomics Space (PTGS) - An Omics-Based Tool for Predictive Toxicity Testing

Kohonen P.[1,2], Nymark P.[1], Hongisto V.[2], Grafström R.[1,2]
[1]Karolinska Institutet, IMM Institute of Environmental Medicine, Stockholm, Sweden, [2]Misvik Biology Oy, Toxicology, Turku, Finland

Predicting possible harmful effects of nanomaterials (NMs) is of great societal concern. We hypothesised that chemicals and drugs induce toxicity mechanisms that are also relevant to NM toxicity, and that toxicity mechanisms occurring in cell cultures might indeed also be predictive for organ pathological states. Accordingly, large data collections of toxicogenomics transcriptomics data and cellular toxicity screening results in cell cultures were subjected to separate and combined modeling and analysis with the purpose of generating a" predictive toxicogenomics space" (PTGS). Such a description was derived by a combination of unsupervised probabilistic modeling with cytotoxicity data to first encapsulate most of the cellular responses to a collection of over 1000 FDA-approved drugs. Subsequently, the toxicity-predictive responses were processed into a novel omics-based toxicity-scoring concept. When tested, a derived PTGS, as well as subcomponents and gene sets, were shown to capture dose-dependent toxicity effects in a variety of published data sets, including the liver, where PTGS covered known pathological states resulting from repeated dosing of rats. Initial assessment relative nanomaterial toxicity-related mechanisms indicated coverage of up to 95% of toxicity mechanisms via subcomponents of the PTGS. Ongoing studies aim at further pinpointing the association of the PTGS to NM toxicity mechanisms relative those induced by other agents. Overall, the novel omics-based tool generated virtual toxicity probability estimates intrinsic to omics data. The scoring concept was shown to broadly capture a diversity of adverse outcomes on cellular and organismal levels in both laboratory animals and humans.

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Raman Spectroscopy as a Potential Technique to Explore the Silver Nanoparticle Eco-Corona Formation

Sekine R.[1], Matzke M.[1], Lynch I.[2], Svendsen C.[1]
[1]Natural Environment Research Council, Centre for Ecology and Hydrology, Wallingford, United Kingdom, [2]University of Birmingham, Geography, Earth and Environmental Sciences, Birmingham, United Kingdom

Background: Nanomaterials are found in many commercial products and this has led to increased concerns regarding their potential risks in the environment. One of the current knowledge gaps in environmental nanotoxicology is in understanding the role of the nanoparticle (NP) surface interactions at the molecular level, particularly in relation to those acquired from the host environment (the eco-corona). This study aims to explore how the eco-corona develops around the NPs using surface-enhanced Raman spectroscopy (SERS) and to examine its impact on the bio-nano interactions.
Material and methods: Citrate stabilized silver NPs were exposed to cytosine and tannic acid, selected based on their known interaction strengths, for 72 h under standard freshwater ecotoxicity testing conditions and were studied with Raman microspectroscopy. Concurrently, dynamic light scattering (DLS) and UV-visible spectroscopy were used to measure their hydrodynamic size and light extinction properties.
Results: DLS and UV-visible spectroscopy indicated that tannic acid and cytosine were physically interacting with silver NPs, implied by the changes in the hydrodynamic size, their extinction profiles and by comparison against the control (without added ligands). SERS confirmed this result and further provided a chemical basis for their interactions that were not revealed by the other methods. Both the concentration and exposure time affected the displacement (of citrate) and attachment of the ligands tested.
Conclusions: SERS has been successfully used to study the molecular interactions at this simple NP system under standard ecotoxicity test conditions, and was shown to complement the data acquired by DLS and UV-visible spectroscopy.