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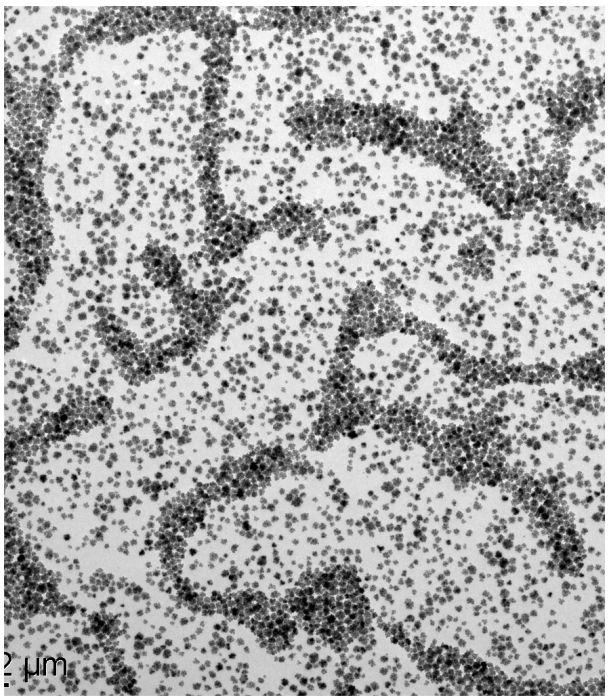

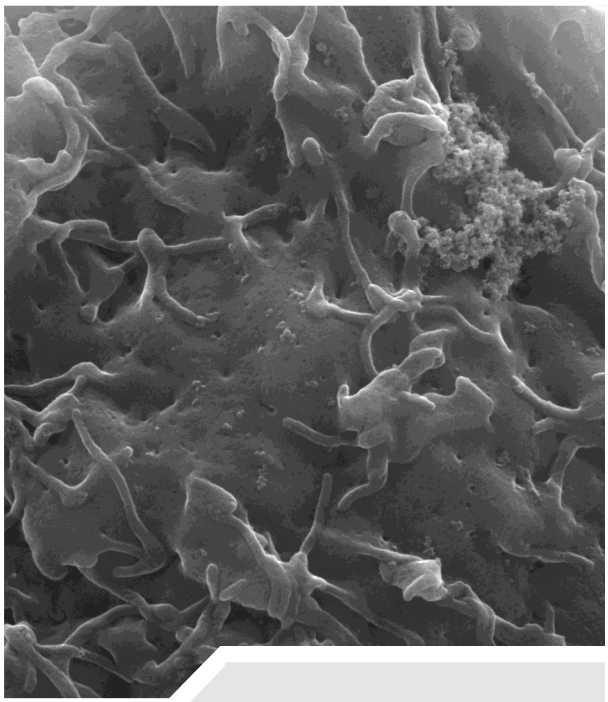

NANOSAFETY 2013

NOVEMBER 20-22, 2013

SAARBRÜCKEN, GERMANY

Leibniz Nanosafety

INM
Leibniz Institute for New Materials

Nanosafety 2013

organised by

INM – Leibniz Institute for New Materials gGmbH

and

Leibniz Research Alliance “Nanosafety”

Saarbrücken, Germany, Nov. 20 – 22, 2013

SCIENTIFIC COMMITTEE

- Eduard Arzt, INM – Leibniz Institute for New Materials, Saarbrücken, Germany, Speaker of Leibniz Network Nanosafety (Conference chair)
- Annette Kraegeloh, INM – Leibniz Institute for New Materials, Saarbrücken, Germany, Scientific coordinator of Leibniz Network Nanosafety (Conference chair)

- Eric Bleeker – RIVM, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
- Heinz Fehrenbach – Research Centre Borstel, Borstel, Germany
- Alexandra K. Kiemer – Pharmaceutical Biology, Saarland University, Saarbrücken, Germany
- Caterina Minelli, Nanoanalysis Group, Analytical Science, National Physical Laboratory, Teddington, United Kingdom
- Klaus Unfried – IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

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- Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund
- Research Centre Borstel – Leibniz Centre for Medicine and Biosciences, Borstel

PROGRAMME

Wednesday, November 20, 2013

08:30 am		Registration and Coffee
09:30 am		Opening Words ▸ Eduard Arzt, INM – Leibniz Institute for New Materials, Saarbrücken, Germany ▸ Susanne Reichrath, State Chancellery of Saarland, Saarbrücken, Germany ▸ Frank Wolf, Federal Ministry of Education and Research (BMBF), Bonn, Germany ▸ Anke Jesse, Federal Ministry of the Environment, Nature Conservation and Nuclear Safety (BMU), Berlin, Germany
		Session 1 NANOOBJECTS: CHARACTERISATION TECHNIQUES AND STANDARDISATION Chair: Tobias Kraus
10:15 am	invited	"Characterisation of Nanomaterials in Regulatory Context" Eric Bleeker, RIVM, National Institute for Public Health and the Environment, Bilthoven, The Netherlands
10:50 am	invited	"Metrology Challenges in the Characterisation of Nanomaterials for Real-World Applications" Caterina Minelli, Nanoanalysis Group, Analytical Science, National Physical Laboratory, Teddington, United Kingdom
11:25 am		"Nanomaterial Characterization Techniques" Ciaran C. Murphy, Malvern Instruments Ltd., Malvern, Great Britain
11:45 am		"'Insight' in Nanomaterial Characterization" Nicole Meulendijks, TNO, Eindhoven, The Netherlands
12:05 pm		"Nanoparticle Tracking and Analysis - the Technology, Applications and its Standardisation" Patrick Hole, NanoSight Ltd., Amesbury, Great Britain
12:25 pm		Lunch break
		Session 2 HUMAN HEALTH: *In vitro*, *ex vivo* AND *in vivo* TESTING (PART 1) Chair: Heinz Fehrenbach
01:30 pm	keynote	"A Strategy to Assess Nanomaterial Toxicity" Thomas Gebel, Federal Institute for Occupational Safety and Health, Dortmund, Germany
02:15 pm	invited	"Membrane-Coupled Signaling Events Elicited by Nanoparticles" Klaus Unfried, IUF-Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
02:50 pm		"TiO_2 but not SiO_2 or ZnO Nanoparticles Accelerate Thrombus Formation in the Murine Microcirculation" Nadine Haberl, Walter Brendel Centre of Experimental Medicine, Ludwig-Maximilians-Universität München, München, Germany

03:10 pm		"New Screening System for Noninvasive Nanotoxicological Real Time Analysis of Single Cells in a Defined Population" Yvonne Kohl, Fraunhofer-Institut für Biomedizinische Technik, Zellbiologie und Angewandte Virologie, St. Ingbert, Germany
03:30		"*In vitro* and *in vivo* Studies to Address the Mechanisms of Pulmonary Toxicity of Multi-Walled Carbon Nanotubes" Catrin Albrecht, IUF Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
03:50 pm		Coffee break
		Session 3 ENVIRONMENTAL IMPACT OF NANOMATERIALS Chair: Eric Bleeker
04:10 pm	invited	"Effects of Nanomaterials in Aquatic Systems" Ralf Schulz , Environmental Sciences, University of Koblenz-Landau, Germany
04:45 pm		"Study of Physical and Chemical Parameters of Submicron and Nanoaerosol in Different Sectors of the Metropolis Anthropoecosystem" Eugeny Kolesnikov, NUST "MISiS", Moscow, Russia
05:05 pm		"Nanosafety Research and Perspectives of Center of Environmental Implication of Nanotechnology (CEIN)" Suman Pokhrel, Foundation Institute of Materials Science, University of Bremen, Bremen, Germany
05:30 – 06:30 pm		Poster Session 1 and Exhibition

Thursday, November 21, 2013

		Session 4 DETECTION AND QUANTIFICATION OF NANOOBJECTS IN LIVING SYSTEMS Chair: Andrea Haase
09:00 am	invited	"Detecting Nanomaterials at the Single Cell Level – Dos and Don'ts" Barbara Rothen-Rutishauser, Adolphe Merkle Institute, University of Fribourg, Switzerland
09:35 am		"Penetration of ZnO Nanoparticles into in vivo Human Skin Studied by Multiphoton Tomography" Hans Georg Breunig, Biophotonik and Laser Technology, Saarland University, Saarbrücken, Germany, and JenLab GmbH, Jena, Germany
09:55 am		"Rapid Examination of Gold Nanoparticle Uptake in whole Cells by Environmental Scanning Electron Microscopy" Diana Peckys, INM – Leibniz Institute for New Materials, Saarbrücken, Germany
10:15 am		"Investigation of Intracellular Nanoparticle Transport and Fate by Image Correlation Spectroscopy" Sarah Deville, Flemish Institute for Technological Research (VITO), Mol, Belgium, and Biomedical Research Unit (BIOMED), Hasselt University, Diepenbeek, Belgium

10:35 am		Coffee break
		Session 5 MODELLING AND PREDICTION OF NANOMATERIAL EFFECTS Chair: Eduard Arzt
11:00 am	keynote	"Small but not Unimportant - Tough to Comprehend and Difficult to Predict: Challenges of Advancing Computational Methods Specific for Nanomaterials" Jerzy Leszczynski, Interdisciplinary Nanotoxicity Center, Jackson State University, Jackson, USA
11:45 am		"Approach to Safe Design of Carbon Nanotubes with Redox Reaction Characteristics" Shuji Tsuruoka, ENCs, Shinshu University, Nagano, Japan
12:05 pm		"Computational Nanotoxicology and Rules for Designing Safer Nanomaterials" Enrico Burello, RAPID, TNO, Zeist, The Netherlands
12:25 pm		Lunch break
		Session 6 SAFETY, CURRENT REGULATION AND SOCIAL/ETHICAL ASPECTS Chair: Alexandra K. Kiemer
01:30 pm	keynote	"Predicting Exposure, Hazards and Risks of Engineered Nanomaterials" Kai Savolainen, Finish Institute of Occupational Health, Helsinki, Finland
02:15 pm	invited	"Overview on Risk Assessment of Nanomaterials under REACH and other European Regulations" Stefania Gottardo, European Commission, DG JRC – Institute for Health and Consumer Protection, Unit I.04 Nanobiosciences, Ispra, Italy
02:50 pm		"Communication about Scientific Uncertainty: How Scientists and Science Journalists deal with Uncertainties in Nanoparticle Research" Ilona Heidmann, University of Koblenz-Landau, Landau, Germany
03:10 pm		"The NANoREG Project and its Novel Approach to Industry Collaboration" Volker Bachmann, Federal Institute for Occupational Safety and Health (BAuA), Berlin, Germany
03:30 pm		Coffee break
03:50 pm		Poster Session 2 + Exhibition
05:00 – 06:15 pm		WORKSHOPS A – "Nanoobjects: Characterisation Techniques and Standardisation" Presenter: T. Müller Opening statements: E. Bleeker, C. Minelli, P. Hole B – "Detection, Quantification and Toxic Effects of Nanoobjects in Living Systems" Presenter: K. Unfried Opening statements: B. Rothen-Rutishauser, A. Haase, R. Schulz C – "Future Needs in Nanosafety" Presenter: H. Fehrenbach Opening statements: T. Gebel, K. Savolainen, K. Wiench
07:00 pm		Conference Dinner: Restaurant 11 ¾, Völklingen (Bus shuttle)

Friday, November 22, 2013

		Session 7 HUMAN HEALTH: *In vitro*, *ex vivo* AND *in vivo* TESTING (2) Chair: Thomas Gebel
09:00 am	invited	“Combining *in situ* Characterization and *in vitro* Toxicity Testing of Nanomaterials” Andrea Haase, Federal Institute for Risk Assessment, Berlin, Germany
09:35 am	invited	“Inflammatory Activation of Cells from the Cardiopulmonary System by Oxidic Nanoparticles” Alexandra K. Kiemer, Pharmaceutical Biology, Saarland University, Saarbrücken, Germany
10:10 am	invited	"The Effects of Carbon Black Nano Particles on Airway Epithelial Cells Differ along the Respiratory Tract" Heinz Fehrenbach, Research Center Borstel, Borstel, Germany
10:45 am		Coffee break
11:00 am		"Impact of the Dynamic Nanoparticle-Protein Corona on Nanosafety" Roland Stauber, Molecular and Cellular Oncology, University Hospital of Mainz, Mainz, Germany
11:20 am		"Toxicogenomic Changes, Oxidative Stress and DNA Damage are Key Factors for Nanoparticles Induced Cellular Anomalies: An Insight into the Molecular Mechanism of Cell Death " Quaiser Saquib, Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
		Session 8 PRESENTATION OF THE WORKSHOPS AND FINAL DISCUSSION Chair: Annette Kraegeloh
11:40 am		Workshop presentations Final discussion Announcement of the Best Poster Award winner Closing remarks
12:40 pm		Light meal
02:00 – 04:00 pm		Guided tour through the INM – Leibniz-Institute for New Materials www.inm-gmbh.de

ORAL PRESENTATIONS

NANOOBJECTS: CHARACTERISATION TECHNIQUES AND STANDARDISATION

O1-01 (invited)

Characterisation of nanomaterials in regulatory context

Bleeker E.A.J.

RIVM, Bilthoven, The Netherlands

In recent years, an increasing number of applications and products containing or using nanomaterials have become available. This has raised concerns that some of these materials may introduce new risks for humans or the environment. A clear definition to discriminate nanomaterials from other materials is prerequisite to include provisions for nanomaterials in legislation. In October 2011 the European Commission published the 'Recommendation on the definition of a nanomaterial', which initiated discussions on how to implement a definition in legislation. In addition, discussions started on specific legal requirements for nanomaterials, most notably on specific information requirements under REACH, but also in relation to e.g. labelling or registering products that contain nanomaterials.

The current status of various regulatory frameworks of the European Union with regard to nanomaterials will be presented, and major issues relevant for regulation of nanomaterials are discussed.

Risk assessment of nanomaterials requires detailed information to characterise the materials. Additional information is needed on the toxic potential of the substances and on their behaviour in humans and the environment, as well as on their fate. The same holds for determining the exposure of humans and environment to nanomaterials and necessary risk management measures to limit the risk. Such assessment of hazards and exposure includes determining the presence of nanomaterials in test systems as well as in the environment, at the work place and other potential areas of exposure. This clearly asks for methodology that enables such measurements. Potential issues that need to be addressed and areas of research in which science can contribute are indicated. These issues include awareness on situations in which nano-related risks may occur for materials that fall outside the definition, guidance and further development of measurement techniques, and dealing with changes during the life cycle.

O1-02 (invited)

Metrology challenges in the characterization of nanomaterials for real-world applications

Minelli C.

National Physical Laboratory, Teddington, United Kingdom

Due to their unique physical properties, engineered nano-scale materials are used to impart enhanced functionality and improved performance to a wide range of consumer products and specialized equipment, such as sunscreen lotions, fuel additives and engineered biosensors. This widespread use of nanomaterials implies increased exposure for humans and the environment through a variety of routes, including production, industrial waste, transportation, usage, storage and disposal.

Currently, enormous efforts are invested worldwide in the development of coherent approaches to the assessment of the impact of nanomaterials on man and the environment, yet knowledge in the field is still limited and standardization of risk assessment methodologies underdeveloped. This complex task faces major challenges, not least the fact that nanomaterials for real-world applications often exhibit non-ideal characteristics, such as broad size distributions, complex or unknown surface chemistry and propensity to dissolve. Furthermore, nanomaterials in a biological or environmental matrix change their surface chemistry as a result of the adsorption of molecules from the surroundings. For example, the protein corona which influences their fate and further interaction with a biosystem.

An increasing number of techniques are today available for the physicochemical characterization of nanoparticles. We recently compared the performance of six particle sizing techniques and highlighted the advantage of the parallel use of complementary techniques to gain an in depth understanding of the materials' properties. This approach proved valuable also in the study of protein-coated nanoparticles. However, many techniques are not suitable for the characterization of nanomaterials with broad size distributions or in complex matrixes. Furthermore, validation of techniques and methodologies is hampered by the lack of well defined, well characterized, readily and widely available reference materials.

O1-03

Nanomaterial characterisation challenges

Kippax P.G., Clarke P.G., Murphy C.C., Kaszuba M.

Malvern Instruments Ltd.

Conservative estimates of worldwide 2010 nanomaterial production volumes would be ~ 270K tons with a market value of ~ $6bn. The production volume in 2016 is conservately estimated to reach ~ 350K driven by demand from applications in electronics, energy, medicine, chemicals, coatings and catalysts. The increasing amount of nanomaterials available in the market creates great opportunities, however it also has the potential for some risk caused by the toxicological effect of these materials when released in the environment.

There has been a large amount of research and investment into the toxicological effects of nanomaterials on the environment and there is – as yet – no definitive findings on the relative weighting and importance of different physico –chemical parameters. However research has indicated that important (nanoparticle) parameters to monitor / consider include size, shape, surface area, surface energy and surface chemistry.

One of the more recent developments in this area is the European Commission definition from 2011 of the term “nanomaterial” . This definition indicates a “Nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for more than 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1nm – 100nm….”, is driving a requirement for higher resolution measurement techniques.

Within this context and background we review some of the latest development activity using light scattering techniques and separations devices aimed at addressing the requirements for number based (quantitative) measurement on Nanomaterials. The work focuses on the characterisation of metallic nanoparticles (silver, gold) and how separation devices (FFF, GPC, Fluidics) can be combined with detectors to offer an improvement in the resolution in line with the demands of the EU nanomaterials definition.

O1-04

„Insight" in nanomaterial characterization

Meulendijks N.[1], Wouters M.[1], Müller T.S.[2], Moh K.[2], Louis C.[3], Amadou J.[4]

[1]TNO, Eindhoven, The Netherlands

[2]INM - Leibniz Institute for New Materials, Optical Materials, Saarbrücken, Germany

[3]Nano-H S.A.S., Saint-Quentin Fallavier, France

[4]Nanocyl SA, Sambreville, Belgium

The In-Sight project is a SME-driven FP7 project on the in-line characterization of nanoparticles during nanomaterial manufacturing. One essential prerequisite for the development, manufacturing and commercialization of nanomaterials is the availability of techniques and tools for real time characterization on the nanoscale level. In the project it is the objective to show that a combination of novel analytical techniques that are capable of real time measurements will provide valuable information for the nanoparticle user and enable monitoring real-time (unexpected) changes in particle count and dimensions during particle processing. The outcome of the project will contribute to minimized batch failure, improved yield, troubleshooting during scale-up. In addition, the in-line measurements will enable 'quality by design' throughout development of new products. Finally, the result of the project will be reflected in a reduction of development time, as well as easy scale-up from lab to manufacturing.

For validation and integration purposes, different off-line analytical techniques were compared using available state of the art analytical tools. Depending on the analytical technique, different sample preparation is often required. As nanoparticles can change structure and composition in response to their environment (i.e. cell growth media), results differ from one analytical tool to another even using the same equipment. In the project, the consortium partners have performed a Round Robin test on off-line characterization of nanoparticles and nanomaterial at their own laboratories. For this purpose, both commercially available standardized particles and complex consortium particles with respect to size distribution and medium have been characterized. In this contribution, we will show the development of novel beyond state of the art in-line monitoring techniques as well as the guidelines for proper nanomaterial characterization.

O1-05

Nano particle tracking and analysis - the technology, applications and its standardisation

Hole J.P., Sullivan J., Sillence K.

NanoSight Ltd., Amesbury, Great Britain

NanoSight has developed a unique capability to directly size and visualize nanoscale particles in liquids (between 10 to 2000 nm), with high-resolution, in real-time and with minimal sample preparation. Nanoparticle Tracking Analysis (NTA) software delivers an unprecedented insight into size distributions through a particle-by-particle approach to particle sizing. With suitable labelling and operation in fluorescence mode, the technology allows targeted particles to be differentiated against complex backgrounds such as biological media. The particle-by-particle Zeta Potential of a sample can be determined by applying an electric field across the cell providing further insight of subpopulations.

Unlike other particle sizing techniques, the data produced undergoes visual validation. NTA can be used to analyze nanoparticles in numerous applications, including drug delivery, nanoparticle toxicology, protein aggregation, exosomes and microvesicles, virology and vaccines, inks and pigments, water purity, and nanobubbles amongst others.

The NTA technology has become rapidly adopted and now has a need for standardisation. To this end various standards are published and others in preparation along with an extensive interlaboratory comparison amongst twelve independent characterisation labs working in the field of toxicology, both to understand the reproducibility and explore additional capabilities of the technique.

It can also be coupled with DLS capability within the same system to extend the size range lower and to an autosampler to enable higher throughput and/or for monitoring temporal changes in e.g. a reaction vessel.

Here we will describe, the technology, its applications, the standardisation work to support it and compare to alternative technologies available.

HUMAN HEALTH: *in vitro*, *ex vivo* AND *in vivo* TESTING

O2/7-01 (keynote)

A strategy to assess nanomaterial toxicity

Gebel T.

baua, Dortmund, Germany

The ‘world’ of nanomaterials comprises an enormous amount of different chemical substances and mixtures. This variability is further increased by the fact that materials with the same chemical identity may appear in different physical forms. All these parameters may have an impact on material toxicology. Complicating the issue, there are toxicological data gaps for nanomaterials which will not be closed in the near future. From a regulatory perspective, all these prerequisites are an immense challenge. This is due to the fact that health risk assessment for each nanomaterial is needed in any situation such material is produced, used and marketed. Thus, approaches to focus are urgently needed. The current knowledge indicates that inhalation exposure is the prominent exposure pathway to be taken into account. Moreover, the possibility to group nanomaterials according to common mode of toxic action offers an advantage with respect to feasibility of health risk assessment. The current toxicological knowledge seems to allow such grouping for respirable biopersistent, fibrous nanomaterials with a certain ‘architecture’ (so-called WHO fibres). Moreover, respirable granular biodurable particles without known significant specific toxicity form a further group of nanomaterials. This latter group comprising nanomaterials like titanium dioxide or carbon black is highly important with respect to marketing volume relevance. For other nanomaterials not belonging to one of these two groups, a risk assessment has to be performed on a case-by-case basis like it is the default situation for all industrial chemicals. In summary, the main challenge in nanomaterial toxicology is mainly dust toxicity. Thus, safety measures should generally focus on avoiding the generation of respirable dust.

O2/7-02 (invited)

Membrane-coupled signaling events elicited by nanoparticles

Unfried K[1], Stöckmann D.[1], Peuschel H.[2], Autengruber A.[1], Gotic M.[3], Kümper A.[1], Engel N.[1]

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[2]INM, Saarbrücken, Germany

[3]Institut Ruder Boskovic, Zagreb, Croatia

Investigating the molecular mechanisms of the interaction of nanoparticles with living cells is of particular interest with respect to nanomedical and biotechnological aspects. But also, with the growing importance of nanotechnology the probability of an unintended human exposure against nanomaterials is exponentially increasing. In addition to directly cytotoxic effects, reactions like apoptosis, proliferation and inflammation may contribute to the toxicity of these materials.

Using carbonaceous particles of different size classes, in our previous studies we identified the initial molecular steps of non-lethal toxic effects of nanoparticles in different types of epithelial cells. Molecular analyses of membrane compartments of alveolar lung cells demonstrate that carbon nanoparticles in lung epithelial cells induce changes in the lipid composition of membrane raft signalling platforms. These molecular events are triggering the ligand-independent activation of the surface receptor EGF-R (epidermal growth factor receptor). Intervention experiments using antioxidants but also biophysically active \'compatible solutes\' demonstrate that the nanoparticle-specific generation of oxidative stress is the initial event of the observed adverse effect and these events can be prevented by stabilization of lipid rafts and macromolecules located within the signalling platforms. Using a set of poorly soluble nanoparticles and their non-nano counterparts, we were able to establish an *in vitro* cell-based test strategy which we suggest as early screening method for the safety of nanomaterials.

Currently the system is evaluated for modern metal-based nanoparticles which are designed for diagnostic and therapeutic applications in humans. Interestingly, in addition to the capability to trigger oxidative stress, properties including particle shape appear to modify the molecular events occurring during nanoparticle cell interaction.

O2/7-03

TiO_2 but not SiO_2 or ZnO nanoparticles accelerate thrombus formation in the murine microcirculation

Haberl N.[1], Hirn S.[2], Holzer M.[2], Zuchtriegel G.[2], Rehberg M.[2], Krombach F.[2]

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[2]Walter Brendel Centre of Experimental Medicine, Ludwig-Maximilians-Universität München, Munich, Germany

Background: It has been suggested that nanoparticles, once arrived in the circulatory system, can interact with the blood coagulation system. The aim of this in vivo study was to investigate the influence of three different engineered nanoparticles, ZnO, SiO2, and TiO2 on thrombus formation in the murine microcirculation.

Methods: C57Bl/6 mice (n=6 per group) were anesthetized and a catheter was placed in the left femoral artery. Then, 1mg/kg BW of ZnO (NM110), SiO2 (NM200) or TiO2 (NM101) NP dispersed in BSA-EtOH-NaCl, according to the NanoGenoTox protocol, were injected. Animals that received only the dispersant served as controls. After 10 minutes, light/dye-induced thrombus formation was assessed in the cremasteric microcirculation by intravital microscopy. Thrombus formation was quantified by determining the time of onset of platelet deposition/aggregation within the microvessel (onset time) and the time required for complete flow cessation for 60 seconds (cessation time) in both one arteriole and one venule per animal. In addition, blood flow velocity, wall shear rate, arterial blood pressure, and heart rate as well as systemic leucocyte and platelet counts were measured.

Results: Injection of ZnO and SiO2 did not have an effect on arterial and venular thrombus formation or on macro- and microhemodynamic parameters. However, injection of TiO2 significantly ($p<0.05$) reduced the cessation time in arterioles to 16.7 ± 4.5 min as compared to controls (40.9 ± 0.7 min) without affecting macro- and microhemodynamic parameters. The onset time in arterioles upon injection of TiO2 was reduced to 8.2 ± 2.2 min as compared to 16.8 ± 3.2 min after injection of vehicle, but did not reach statistical significance. In contrast, injection of TiO2 did not affect venular thrombus formation.

Conclusion: These findings indicate that TiO2 but not ZnO or SiO2 nanoparticles, when injected into the blood circulation, accelerate thrombus formation in murine arterioles.

O2/7-04

New screening system for noninvasive nanotoxicological real time analysis of single cells in a defined population

Kohl Y.[1], Shah A.[1], Knoll T.[1], Gorjup E.[1], Duschl A.[2], Briesen H.[1]

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[2]University of Salzburg, Salzburg, Austria

With the increasing use of nanomaterials (NMs) in consumer and medical products, the need for risk assessment and new methods to examine their safety is becoming urgent, in particular for NMs that are deliberately used in medicine, cosmetics and food.Existing standardized toxicological *in vitro* assays mostly average the response of a complete cell population. Such averaging might result in misinterpretations due to the masking of individual cell variations. NM-induced cell response may vary from cell to cell. Thus, there is a demand for new *in vitro* systems for a sensitive noninvasive long-term characterization of individual reactions on cellular level to obtain more detailed information on the toxicity of these materials.

In the present study a lens-less imaging system (LESY) was developed and validated for noninvasive real-time investigation of nanoparticulate effects on the cellular level.

By semiconductor process technology a micro cavity chip (MCC, culture area < 0.3 μm^2) with a transparent silicon nitride membrane (thickness 800 nm) was established. Biocompatibility of the MCC was determined by analyzing specific characteristics of different human cell lines (stem cells, neuronal cells, lung cells, GFP-transfected reporter cells) after culturing and differentiating in the MCC. Time- and dose-dependent effects of NMs on differentiation and inflammation of individual cells could be shown successfully.

The MCC was merged with optical and fluidic technology as miniaturized MCC-based LESY. The new developed system was validated as a miniaturized cell culture incubator with optical characterization techniques for permanent tracking of individual cell response. In the compact LESY cell culturing up to 16 days was successful.

Thus the developed MCC-based LESY is suitable as a new screening system for noninvasive nanotoxicological real time analysis of single cells in a defined population and is one approach for risk assessment of NMs within the framework of REACH.

O2/7-05

In vitro and *in vivo* studies to address the mechanisms of pulmonary toxicity of multi-walled carbon nanotubes

Schins R.P.F.[1], Boots A.W.[2], van Berlo D.[3], Wilhelmi W.[1], Hullmann M.[1], Kuhlbusch T.[4], van Schooten F.J.[2], Bast A.[2], Albrecht C.[1]

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[3]Division of Biological Stress Response, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[4]IUTA e.V., Air Quality & Sustainable Nanotechnology Unit, Duisburg, Germany

There is an increasing concern about the safety of multi-walled carbon nanotubes (MWCNT). Studies in rodent models have shown that these high aspect ratio nanomaterials can cause pulmonary inflammation and fibrosis. In a series of studies we have investigated the potential underlying mechanisms. In a first study, mice were exposed by pharyngeal aspiration to two types of MWCNT, characterized by a contrasting average tube length and entanglement. The longer MWCNT triggered a more pronounced pro-fibrotic response, shown by stronger Masson trichrome staining and increased mRNA expression of MMP-8 and TIMP-1. Enhanced apoptosis in the MWCNT treated mice was detected by cleaved caspase 3 immunohistochemistry. However, this staining was merely localized to granulomatous foci. Parallel *in vitro* studies in mouse RAW264.7 macrophages revealed increased cytotoxicity only with the longer CNT. Apoptosis, evaluated by a caspase 3/7 activity assay, was not observed in these cells. To address the role of reactive oxygen species formation, the effects of long type MWCNT were subsequently evaluated in mice lacking the redox sensitive transcription factor Nrf2. The pro-oxidative effect of the MWCNT treatment was demonstrated by a decreased pulmonary total antioxidant capacity in the Nrf2-deficient mice compared to their wildtype counterparts. As anticipated, the lungs of the MWCNT treated knockout animals also failed to show a compensatory upregulation of the expression of the Nrf2-regulated enzymes HO-1 and gamma-GCS. Remarkably, however, no major deterioration in pulmonary damage could be observed in the Nrf2 deficient mice in comparison to the wildtype animals. Our investigations confirm that MWCNT can trigger pro-fibrotic responses in murine lungs to an extent that depends on their physicochemical properties. Our data also reveal that this effect may not critically depend on the functionality of the major cellular redox regulator Nrf2.

O2/7-06 (invited)

Combining *in situ* characterization and *in vitro* toxicity testing of nanomaterials

Haase A.[1], Driessen M. D.[1], Ossig R.[2], Schnekenburger J.[2], Vennemann A.[3], Wiedmann M.[3], Lehr C.-M.[4], Luch A.[5], Wohlleben W.[6]

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[4]Helmholtz Institute for Pharmaceutical Research, Saarbrücken, Germany

[5]German Federal Institute for Risk Assesssment, Berlin, Germany

[6]BASF SE, Ludwigshafen, Germany

The BMBF-funded project "nanoGEM" follows a systematic approach to understand hazards associated with different types of nanoparticles (NP). Oxidative stress is considered to be a major paradigm to explain NP toxicity. Here we focused on protein carbonylation as a consequence of oxidative stress for a set of 16 different nanoparticles.

We used NP of 10nm (ZrO2), 15nm (SiO2) and 50nm or 200nm (Ag), furnished either with acidic, basic or polymeric functionalities and TiO2, ZnO, BaSO4 and AlOOH as references. In a screening approach we studied time- and dose-dependent carbonylation of all 16 NP in NRK-52E cells via 1D immunoblots. Data were correlated with cytotoxicity (WST-8, LDH assay) and ROS formation (DCFDA assay). Furthermore we applied a 2D proteomics approach combined with MALDI-MS/MS to identify the modified proteins. Finally for several NP we analyzed lung tissues after in vivo instillation in rats.

Eight out of 16 NP induced carbonyls in NRK-52E cells, which is in good correlation with overall toxicity. The 2D approach revealed a complex and distinct pattern of carbonyls. Modified proteins were identified as proteins of cytoskeleton, HSP or proteins of major cellular pathways (i.e. glycolysis). We also observed carbonyl modifications in the lung tissue homogenates of rats intratracheally instilled with Ag NP. Analysis of protein carbonylation appears useful to describe specific effects of NP and to better understand the molecular mechanisms underlying NP toxicity.

Furthermore, we analyzed agglomeration as well as lipid and protein interactions of all 16 NP in serum containing cell culture medium and in native lung surfactant (nS). In protein containing media, only NP with electro-steric functionalities remained dispersed, partially due to negative charge; all NP attracted a corona. Phospholipids had a low affinity to NP. Only few surface functionalities attracted lipids, which then led to subsequent agglomeration of NP. Interestingly, the presence of surfactant proteins in nS seemed to mediate lipid binding in some cases but not vice versa.

Taken together NP behave different in nS compared to serum containing cell culture media. This should be taken into account for *in vitro* toxicity testing especially if lung derived cell lines are used.

O2/7-07 (invited)

Inflammatory activation of cells from the cardiopulmonary system by oxidic nanoparticles

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Inflammatory processes are involved in the development of diverse pathologies in the cardiopulmonary system, such as chronic obstructive pulmonary disease (COPD) or atherosclerosis. Numerous *in vitro* and in vivo data report that exposure to technical nanoparticles can induce inflammatory reactions (1, 2). Still, a detailed understanding of the cellular mechanisms involved is widely lacking. Examples will be presented how oxidic nanoparticles can promote inflammatory cell activation by either (I) increasing the production of inflammatory mediators or (II) by decreasing the production of anti-inflammatory mediators.

The first part of the talk is dedicated to the activation of primary human alveolar macrophages by nanoparticulate silica. The lung represents a major port of entry for engineered nanoparticles. Since alveolar macrophages are in direct contact with the ambient air they represent the first line of defense against inhaled particles and infectious agents.

The second part of the talk will focus on the effects of superparamagnetic iron oxide nanoparticles (SPION) on endothelial cells as the cell type lining the vasculature. SPION have been used for diagnostic purposes for many years and are under development for diverse diagnostic and curative applications. Since the particles are typically injected into the blood stream, the interaction of SPION is of high practical relevance.

Taken together, the talk will highlight mechanistic findings regarding the effects of technical nanoparticles on cells of the cardiopulmonary system. Respective interactions are suggested to be highly relevant for occupational health, diagnostics, and therapy.

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O2/7-08 (invited)

The effects of Carbon Black nanoparticles on airway epithelial cells differ along the respiratory tract

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The worldwide production of Carbon black nanoparticles (CBNP), which are mainly used for reinforcement of rubber and plastic material, amounts to several mega tons per year. During synthesis and manufacturing CBNP can adsorb diverse substances at their surface. In order to assess the contribution of functionalized surfaces to potential biological or toxicological effects of CBNP on airways and lungs, a test system was used which is composed of several models with increasing complexity.

Gas-flame synthesized CBNP were modified with respect to the presence of polycyclic aromatic hydrocarbons (PAH) and substituted PAH on their surface. Reference and functionalized particles as well as CBNP suspensions in albumin containing buffers were characterized in detail (e.g. by GC/MS, DRIFT, TGA/MS, BET, HR-SEM, DLS) and delivered as stock material to the participating biological test laboratories. Concentration ranges of CBNP of 0.1 up to 100 µg/ml were assessed in lung and airway epithelial human cell lines as well as in precision cut murine lung slices, in explanted mouse trachea as well as in proximal and distal intrapulmonary airway explants *in vitro*. In addition, CBNP were assessed in primary murine alveolar epithelial cells *in vitro* and in mouse lungs after oropharyngeal aspiration of CBNP. In vivo inhalation experiments are currently performed.

Whereas several readouts such as necrosis, apoptosis, oxidative stress and cytokine production were analysed in each test system, other parameters such as transepithelial electric resistance (cell lines), ciliary beat frequency (trachea), xenobiotic metabolism (intrapulmonary airways) or surfactant metabolism (alveolar epithelial cells) were measured in specific lung compartments only.

Our results suggest that there are differences in the, albeit mild, toxicological and biological responses to CBNP that depend both on the lung compartment studied and the chemical nature of the CBNP surface.

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O2/7-09

Impact of the dynamic nanoparticle-protein corona on nanosafety

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Nanomaterials adsorb biomolecules upon contact with all biological environments. Therefore, the biomolecules-coated nanomaterials may need to be considered as ‚new materials' compared to the pristine nanomaterials during their manufacturing. Particularly, the so called "nanoparticle-protein corona" is expected not only to critically impact nanotoxicology and nanoecology but also influences the success and safety of nanobiomedical applications. As most biological systems are (highly) dynamic, also a time-resolved knowledge of particle-specific protein fingerprints is required to understand the coronas' evolution, enabling predictions, prevention or rational enforcement of nanoparticle-induced (patho)physiological effects, affecting nanosafety.

Employing label-free liquid chromatography mass spectrometry, we present not only a qualitative but also a quantitative systematic analysis of the human blood protein corona on nanoparticles varying in distinct physico-chemical features. Our results provide novel insights into the complexity and kinetic evolution of particle-specific protein signatures. Collectively, we demonstrate that already the rapid corona formation is (patho)biologically relevant and provide bioinformatic potential risk predictors. Combined with comprehensive cell-based (high-throughput) assays, the impact of corona evolution as well as its rational exploitation for advanced nanomaterial with improved safety will be discussed.

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O2/7-10

Toxicogenomic changes, oxidative stress and DNA damage are key factors for nanoparticles induced cellular anomalies: An insight into the molecular mechanism of cell death

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This study is based on the rationale to provide concrete evidence on the cross-talk between toxicogenomic changes, oxidative stress, DNA damage and cell death induced by CoO-NPs and ZnFe2O4-NPs in HepG2 and WISH cells. Ultra-structure analysis revealed that NPs were translocated into the cytoplasm and induce DNA damage. MTT and NRU assays showed cytotoxicity of 26.4%, 27.06% in HepG2 and 18.4%, 50.73% in WISH cells. Intracellular ROS analysis in CoO-NPs and ZnFe2O4-NPs treated cells exhibited 1.12 and 1.2-fold higher ROS level along with change in membrane potential of mitochondria. Comet assay reveled 2.6 and 7.4-fold higher DNA damage in HepG2 and WISH cells at the highest concentration of 100 μg/ml. Flow cytometric analysis of CoO-NPs treated HepG2 cells exhibited G2/M arrest with 23.36% of cells vis-à-vis the control showed only 17.3% of cells in G2/M phase. On the other hand WISH cells exhibited apoptotic response with 15.2% cells in subG1 phase upon ZnFe2O4-NPs treatment. qPCR analysis of P53 and caspase 3 genes in HepG2 cells showed 1.3 and 1.0-fold higher expression by CoO-NPs. Comparatively, WISH cells also exhibited 5.3, 1.6, and 14.9-fold upregulation of P53, caspase 3 and bax genes. The RT^2 Profiler™ PCR array data of 84 genes responsible for oxidative stress and human toxicity elucidated up-regulation of mRNA transcripts of UNG, CDKN1A, GDF15, MDM2, IGFBP6, NFKBIA, TNFRSF1A, TNFSF10, MIF, NOS2, HSF1, HSP90AA2, HSPB1, PRDX1 and CYP1A1 genes in range of 1.2 to 3.0-folds in HepG2 cells. Also, WISH cells treated with ZnFe2O4-NPs elucidated differential up-regulation of CCL21, NFKB1, IL-1b and NOS2 genes in range of 1.5 to 3.7-folds. In conclusion, significant ROS production, reduction in ΔΨm, DNA damage, and activation of genes linked to inflammation, oxidative stress, proliferation, DNA damage and repair could serve as predictive toxicity and stress markers for toxicological assessment of NPs induced cellular and genetic damage in exposed human population.

ENVIRONMENTAL IMPACT OF NANOMATERIALS

O3-01 (invited)

Effects of Nanomaterials in Aquatic Systems

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The increasing production and use of enginereered nanomaterials inceases their chance to enter non-target environments such as surface waters. This raises questions as to the risks they may pose to aquatic organisms. Whether nanomaterials excert any harmful effects in the aquatic environment will greatly depend on their characteristics, on the environmental conditions and on ecological features. Most aquatic studies have assessed titanium dioxide nanoparticles (nTiO2) effects on on the water flea Daphnia magna, a planktonic crustacean regularly used in ecotoxicity testing. Effect thresholds vary over several orders of magnitudes, many studies have not characterised the particles used and thus we sincerely lack an understanding of the mechanisms underlying nanoparticle toxicity. This presentation provides examples on test design and endpoints to be used to provide understanding for ecotoxicological processes responsible for adverse effects. Though the effect threshold concentrations still are rather high, recent studies point even for compounds such as nTiO2, which do not show any noteworthy classical toxicity, in the direction of ecological meaningful effects at concentrations levels that need to be considered in a risk assessment context.

O3-02

Study of physical and chemical parameters of submicron and nanoaerosol in different sectors of the metropolis anthropoecosystem

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The dynamics of the existing industrial technologies associated with the production of highly dispersed technological waste (metallurgy, petrochemical, mining and processing of mineral resources, the automotive industry, etc.), and even more manufacturing application of nanotechnologies and nanomaterials, significantly ahead of our knowledge in the field of security their manufacture and use. In this regard, the question of environmental monitoring for the presence of sub-micron and nano-sized particles in air, water, solid and liquid industrial waste, the development of methods and means of protection, control and disposal becomes relevant [P. Houdy et. al. 2010].

The aim of this work was to monitor the concentration and the study of physical and chemical parameters of submicron and nanoaerosol in a large metropolis.

The measurements were obtained with the use of a Scanning Mobility Particle Sizer Spectrometer 3936 (TSI Inc). Elemental composition and morphology were studied using TEM (JEOL JEM-1400) and scanning electron microscopy with energy dispersive X-ray analyzer (JEOL JSM-6610).

The study was performed in Moscow (the largest city in Europe, the population in 2013 of about 12 million people). Three model territory of the city, with varying degrees of anthropogenic load: park, residential zone, industrial zone have been identified.

According to a study set characteristics of aerosol particles lying in the sub-micron and nanometer scale. Typical elemental composition and concentration were determined.

O3-03

Nanosafety research and perspectives of Center of Environmental Implication of Nanotechnology (CEIN)

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Fundamental understanding of nanomaterials in living organisms and the environment is vital to understand their safety. The combined effort of the working groups under the umbrella of University of California Center for Environmental Implications of Nanotechnology (UC CEIN, USA) uses a multidisciplinary approach to conduct research and develop paradigms for safe use of nanotechnology in the environment. The various nanoparticle libraries such asmetal oxide are designed/engineered, through alteration of physicochemical properties in determining environmental fate, transport, exposure, and hazard generation across a broad spectrum of bio-nano interfaces in cells, bacteria and other higher organisms. These well characterized combinatorial libraries undergo high throughput screening (HTS) approaches for developing relationships between the nanoparticle properties and their effect (structure-activity relationship (SAR)) in the cellular media.2 Additionally, these libraries are also exploited to determine their fate and transport along with the materials bioavailability. The data acquired from the entire center undergo In silico transformation involving data integration to provide hazard ranking, exposure modeling, risk profiling, and construction of nano-SARs. The knowledge evolved from the CEIN research activities are transferred within the educational institutes, as well as industries. The main focus of the center is to make use of the benefits of nanotechnology towards global economy, society and the environment.

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DETECTION AND QUANTIFICATION OF NANOOBJECTS IN LIVING SYSTEMS

O4-01 (invited)

Detection of nanomaterials at the single cell level – must and don'ts

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With the ever increasing production and use of engineered nanomaterials it is crucial that the interaction of such nano-sized materials with biological systems is understood. In contrast to the extensive literature available on the synthesis and physicochemical properties of nanomaterials, information concerning their fundamental biological interactions remains fragmented at best. Understanding the interaction of nanomaterials at the single cell level, their uptake and intracellular trafficking can provide essential information pertaining to the potential reactivity of any nanomaterials.

Due to the small size of nanomaterials their identification and localisation within cells is extremely challenging. Therefore, various cutting-edge techniques are required to detect both fluorescent and electron dense nanomaterials such as fluorescence-activated cell sorting (FACS) analysis, Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES), laser scanning microscopy (LSM) as well as transmission electron microscopy (TEM) techniques such as energy loss spectroscopy and electron tomography. An overview will be given regarding advanced material synthesis approaches as well as the application of the above mentioned methods including a thorough discussion about limitations and pitfalls of each of the techniques.

O4-02

Penetration of ZnO nanoparticles into *in vivo* human skin studied by multiphoton tomography

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We report on the detection, identification and even quantification of nanoparticle distributions on human skin in vivo by multiphoton tomography, a non-linear optical microscopy technique. Exemplarily, we have studied the penetration of zinc oxide (ZnO) nanoparticles, commonly used as UV filters in commercial sunscreens, into human skin. Multiphoton tomography relies on ultrashort infrared pulses which are focused and raster scanned over the imaging region. The resolution depends on the focal volume and is typically about 500 nm laterally. The laser pulses generate characteristic nonlinear optical signals like second harmonic generation and hyper-Rayleigh scattering by interaction with the ZnO nanoparticles. By detecting these signals, the distribution of ZnO nanoparticles can be imaged even though the size of the nanoparticles is smaller than the optical resolution. Skin-tissue autofluorescence is spectrally well separated from the signals of the nanoparticles and simultaneously recorded to obtain sub-cellular resolution skin images. The nonlinear nature of multiphoton imaging also provides intrinsic depth resolution and 3D-imaging capability. For in vivo imaging of human skin the imaging depth is limited to 200 µm. We found that ZnO nanoparticles (with a size of 30 nm) penetrated only into the outermost layers of the stratum corneum, skin furrows and into orifices of hair follicles but did not reach lower lying skin layers of the epidermis. For the particulate ZnO nanoparticles a detection limit of 0.08 fg/µm^3 on the skin could be estimated.

O4-03

Rapid examination of gold nanoparticle uptake in whole cells by environmental scanning electron microscopy

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Gold nanoparticles (AuNPs) find broad usage as markers in biomedical research and for cancer therapy. Quantitative knowledge about their long-term intracellular fate is essential for their safe usage in humans. We have developed a fast high-resolution electron microscopy (EM) method to study and quantify AuNPs in whole cells, by using a sample preparation method similar to those used in light microscopy. Imaging was done on fixed, intact cells, kept in a water vapor atmosphere under a thin water layer, with an environmental scanning electron microscope (ESEM) equipped with a scanning transmission electron microscope (STEM) detector. This method allows resolving individual, intracellular AuNPs in whole cells.

In this particular study, we tested the influence of the AuNP diameter on its long-term intracellular storage process. Lung cancer cells (A549) were grown on electron transparent microchips and incubated with serum protein coated AuNPs for 2 h, followed by 22 or 43 h incubation without AuNPs of either 10, 15, or 30 nm.

At both examined time points, the AuNPs were found in vesicles that were randomly scattered throughout the cytosol. The amount of intracellular AuNPs-filled vesicles varied between the cells. The intravesicular distribution of the AuNPs suggested their attachment at the vesicle membrane, indicating the persistence of their protein corona. 145 cells were imaged and the size of 1,041 AuNP containing vesicles was determined, revealing an average size of 490 nm for vesicles containing 30 nm-diameter AuNPs, but 80 - 70 nm smaller sizes for vesicles with 10 or 15 nm AuNPs ($p < 0.001$, Student's t-test). The entire study was completed in only about 80 h; this is by a factor of 50 – 70 faster compared to conventional electron microscopy studies. Note, that different types of nanoparticles, for example, made of silicon, can also be detected using this method, but a higher electron dose is needed the lighter the material of the nanoparticles is.

O4-04

Investigation of intracellular nanoparticle transport and fate by image correlation spectroscopy

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The intracellular transport of nanoparticles (NPs) is a key event of interest to both nanotoxicology and nanomedicine. Once inside, a multitude of intracellular targets is available which can provoke a biological response upon interaction with NPs. In the present study, 40-nm size fluorescently dyed carboxylated polystyrene (PS) NPs were used to provide insights into the intracellular NP dynamics using the human alveolar epithelial A549 cell line. PS NPs are commonly used as model particles to study interaction with biological systems due to their commercial availability, high quality and wide variety of size and surface chemistry. Temporal and spatio-temporal image correlation spectroscopy (TICS and STICS) were performed to investigate the NP motions inside the cell. Using these quantitative imaging techniques, NP fluorescence intensity fluctuations within a microscopy image series were measured for characterization of the intracellular transport direction, transport velocity and diffusion following NP uptake. NP motions exhibited a strong directed flow, suggesting an active transport component. Potential dynamic interactions of NPs with the nucleus, mitochondria, early endosomes, late endosomes and lysosomes were further explored with spatio-temporal image cross-correlation spectroscopy (STICCS) using organelle specific dyes. Here, the space-time cross-correlation between the images collected in the NP channel and the organelle specific channel was evaluated. PS NPs were found to be predominantly associated with the endolysosomal compartment and the mitochondria. In summary, we provided deeper insights in PS NPs' intracellular motion and fate in A549 cells. Moreover, we demonstrated the ability of image correlation-based methods to study the dynamic processes of NP transport within the cell.

MODELLING AND PREDICTION OF NANOMATERIAL EFFECTS

O5-01 (keynote)

Small but not unimportant - Tough to comprehend and difficult to predict: Challenges of advancing computational methods specific for nanomaterials

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Nanotechnology has emerged at the forefront of science and technology developments. This is due to the fact that nanoparticles (NPs) have a wide range of applications in different aspects of human life. However, there is also the other, disturbing side of fast advances in nanotechnology. This side is often hidden by companies which are only concern in increasing their short term profits. Therefore, it is a crucial role of scientists to uncover potential side effects of nanoparticles, inform the public, and provide solutions to the possible problems. The truth is that due to unique properties of NPs they could be harmful to the environment and humans.

There are various experimental techniques that are used to study different properties of nanomaterials, including their toxicity. However, such techniques are expensive to use and time consuming. There is a necessity to develop alternative methods, easy to use, fast, and efficient. Computational chemistry provides diverse tools that could evaluate molecular interactions among various species including nanoparticles and models of different biological species, and predict their properties and biological activities. Novel European regulations named REACH System (Registration, Evaluation Authorization of Chemicals), strongly promotes application of computational methods to evaluate properties and biological activity of new species: \"In particular for human toxicity, information shall be generated whenever possible by means other than vertebrate animal tests, through the use of alternative methods, for example qualitative or quantitative structure-activity relationship (QSAR/QSPR) models...\". The talk will be devoted to discussion of new challenges that nanomaterials create for the society.

A part of the lecture will cover development of novel computational approaches, appropriate for evaluation of properties and activities of nanostructures. Current status of Nano-QSAR models will be discussed. The obtained results could be used as a first step in developing mechanisms that explain complex interactions of nanomaterials with biomolecules.

O5-02

Approach to safe design of carbon nanotubes with redox reaction characteristics

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Carbon nanotubes (CNTs) are becoming important materials in industry and are expected to make significant contributions to benefit human society. It has become an important goal recently to design safe CNTs by controlling their physicochemical properties and thus their bioactivity. Although CNTs are known as ROS scavengers, this property depends on surface morphology. To date, there is a lack of information to describe a relationship between the physicochemical properties of CNTs and their bioavailability. We hypothesize that CNT surface chemical reactivity with dangling bonds plays an important role in determining bioactivity. The present work investigated factors that disturb ROS measurement using the ESR-DMPO method to determine radical concentrations in the presence of CNTs. Radical degeneration rate by CNTs was measured in a minimal concentration of surfactant to avoid its influence on the assay, since results show that surfactant can affect ROS measurement and confound studies to characterize the scavenging action of CNT surface morphology. Use of minimal surfactant allowed evaluation of the relationship between ROS quenching and the amount of surface defects of CNTs or the amount of dangling bonds on the carbon structure. It was found that the radical reaction on the CNT surface is described in a stoichiometric manner with its reaction kinetics. Thus, as the quenching is apparently a redox reaction, the result demonstrated that CNT morphology affects bioavailability. Results suggest that it is possible to design safe CNTs considering their redox potential and surface morphology.

O5-03

Computational nanotoxicology and rules for designing safer nanomaterials

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A comprehensive evaluation of the biological and toxicological effects of nanomaterials is necessary for the design of products that are safe and operate as intended. However, toxicological tests are time-consuming and resource-intensive, which is why researchers are developing computational models to predict the behavior of nanomaterials in biological systems. Such predictions would contribute to streamline and prioritize toxicological tests on real nanomaterials as well as to support safe design and risk assessment strategies.

Here, we present 4 models for profiling the potential biological and toxicological effects of oxide nanomaterials. These models attempt to describe the reactivity, protein adsorption, membrane adhesion and biopersistence processes of a large number of oxide nanomaterials and are based on physicochemical properties calculated from experimental data or obtained by statistical regression methods. The proposed models are then used to derive safe design rules and to develop a conceptual framework for risk assessment of nanomaterials.

SAFETY, CURRENT REGULATION AND SOCIAL/ETHICAL ASPECTS

O6-01 (keynote)

Predicting exposure, hazards and risks of engineered nanomaterials

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The use of engineered nanomaterials (ENM) grows rapidly. Today, about 1-3 million workers are exposed to ENM at workplaces, and the number will be about 10-15 million by 2020. In parallel, exposure of consumers and the environment is also on an increase. This emphasizes the importance of reliable and predictive assessment of exposure to and hazards of ENM; it is a prerequisite for reliable risk assessment and management. In fact, only very few ENM have been systematically studied for hazards and exposure in a way that would allow ENM-related knowledge-based risk assessment and management. Several experimental studies have though increased our understanding on the toxicity mechanisms of ENM, but toxicity and exposure data on ENM supporting regulations and regulatory decision making is scanty. An additional challenge is that there is not yet a consensus on the metrics of ENM to be used in assessing exposure of defining dose for toxicity studies. However, both pieces of ENM-related information are required for reliable and predictive risk assessment of ENM. Current risk assessment is based on the more than 30 year's old risk assessment paradigm, or its modifications, that include 1) hazard identification; 2) hazard assessment; 3) exposure assessment; and 4) risk assessment. This concept is laborious and too expensive to enable risk assessment of ENM on the market and entering the market. In the future, the utilization of omics technologies, systems biology approaches and bioinformatics are increasingly important. Supported by EU Commission Grant CP-IP 211464-2 (NANODEVICE).

O6-02 (invited)

Overview on risk assessment of nanomaterials under REACH and other European regulations

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In the EU the safety of nanomaterials (NM) is regulated by a legal framework, which implicitly or explicitly (recent revisions) addresses NM. NM are implicitly covered by the substance definition of REACH Regulation 1907/2006. Under REACH a registration dossier has to be submitted if a substance is produced/imported in volume higher than 1 ton/year and a Chemical Safety Report has to be prepared if the volume is higher than 10 ton/year. The registrant can either explicitly cover the nanoform in the dossier of the bulkform or submit a specific dossier for the nanoform. Regardless of tonnage, NM have to be classified for dangerous properties (CLP Regulation 1272/2008). NM are also regulated by sector-specific legislation (cosmetic products, food, biocidal products), which include explicit provisions for NM such as a definition of the term \'nanomaterial\', separate assessment of their risk, labelling/reporting requirements. An EC Recommendation (2011/636/EU) on the definition of the term \'nanomaterial\' for regulatory purposes was adopted in 2011 and will be revised by 2014.

Several expert bodies agree that the existing risk assessment procedures are applicable to NM provided that some adaptations are considered to address their peculiarities. The present knowledge does not allow generalisations on the risk caused by NM and therefore a case-by-case approach is currently recommended. Guidance for NM risk assessment is available for industrial substances, food and cosmetic products. The EC is also investigating the possibility of including information requirements for NM in the REACH Annexes. In addition, specific working groups have been established to develop best practices of NM safety assessment and to facilitate harmonisation of assessment methodologies. A challenge for the future is the development of tiered approaches as well as grouping/waiving options to optimise testing and properly address the risks posed by NM.

O6-03

Communication about scientific uncertainty: How scientists and science journalists deal with uncertainties in nanoparticle research

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'There is a high level of scientific uncertainty in nanoparticle research' is often stated in the scientific literature, e.g., concerning the environmental fate of nanoparticles. Knowing more about these uncertainties and the communication about it in scientific literature and mass media might be of interest to other scientists and experts. Due to this, we compare the current state of scientific knowledge about scientific uncertainty through the example of environmental nanoparticle research with the media coverage in the field of nanotechnologies.

In research and review papers, scientific uncertainties, sources, and consequences are mentioned with different foci and to a different extent. In research papers, the authors focus on the certainty of specific results, whereas in review papers, the uncertainties due to a general lack of data are emphasized and the sources and consequences are discussed. The content analysis of the media coverage shows that nanotechnology is often framed in political discourses and as rather certain, and only one-third of the reports deal with scientific uncertainties. Furthermore, there is a strong relationship between the representations of scientific uncertainty and risks. Environmental issues are seldom mentioned.

Scientific uncertainties, sources, and consequences have been most widely discussed in the review papers. Research papers and mass media tend to emphasize more the certainty of their results or topics. Neither the broad spectrum nor any specifications of uncertainties have been communicated. This indicates that there has been no effective dialogue over scientific uncertainty with the public so far.

O6-04

The NANoREG project and its novel approach to industry collaboration

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The research project “A common European approach to the regulatory testing of nanomaterials” (NANoREG) is the EU FP7 flagship project in the field of ensuring the safety of nanotechnology. By reviewing the testing methods in current regulation for their applicability to nanomaterials it aims to accelerate the regulatory process. An overview of the project and first results will be given.

Industrial innovation and product development does not wait for regulatory mechanisms to be developed. NANoREG is developing new paths integrating risk analysis as part of the innovation process of products. NANoREG\'s processes contribute to the safe use of manufactured nanomaterials entering in the market. This concept aims at creating an integrated research strategy to maximize resources and expedite the development of nanomaterials that are safe by design. It closes the gap between innovation and safety research and will foster a better collaboration between stakeholders, due to better insight in pieces of common interest at an early stage.

To make NANoREG a success the collaboration with industry is essential. For an enterprise or association there are three ways to participate in this project: 1. as Interested Industry and Industry Association, 2. as Member of the NANoREG Industry Consultation Committee, or 3. as project partner in a Value Chain Project (VCP) or Working Group (WG). Depending on the kind of participation an enterprise or association can communicate the industry’s needs in regulation to NANoREG. NICC members will be asked to give advice to the project. They can initiate topics for VCPs or WGs and implement such studies by using real cases from their daily business. By providing reliable and relevant data industry can contribute to the results of the project and to the formulation of workable answers to industries needs and questions from the regulators. The benefits and conditions of industry participations will be explained in detail.

POSTER

NANOOBJECTS: CHARACTERISATION TECHNIQUES AND STANDARDISATION

P1-01

Investigation of rheological and mechanical behaviour of LDPE with micro and nano-carbon black particles

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This work focuses on studying the characteristics of LDPE reinforced with micron and nano carbon black particles. The LDPE melt blended with 2% , 4% and 8% of micron and nano carbon black respectively in a co-rotating twin screw extruder. different testing equipments were used in this study(tension, impact, DSC,PSA,....etc)also, MFR has measured using melt indexer type (SHI JIA ZHUANC ZHONG SHI TESTING MACHINE CO., LTD). The results show that the best characteristics for the composite materials within the lower percentage of NCB and best of the micron CB composite materials. MFR reduced with micron and nano CB concentration and increased with the temperatures increasing, the MFR and shear rate at the wall exhibit lower values with micron CB composites than that of nano CB composites.

P1-02

Project InSight

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The Project InSight (Real time In-line characterization of nanoparticles) is an FP7 project targeting the development of new tools for real time monitoring and characterization of Nanoparticles. It is executed by a consortium of 8 SME companies and 2 research centers. It intends to develop a unified system with instruments determining the shape and mobility, the number and size as well as the composition of nanoparticles. The instruments are intended for application like the synthesis of nanoparticles or the manufacture of nanomaterials, but also analytical applications in a scientific environment are purposed.

These instruments utilize optical and acoustical detection principles for measuring these properties in an on-line or in-line fashion instead of the off-line measurements normally used. After 24 months of the projects term stand-alone instruments were manufactured as working prototypes. During the last 12 months of the project an integration of the different stand-alone instruments shall be archived via a uniform front end for operating the system with selectable modules for the methods.

The novel tools shall be validated against existing standard methods, as a base for these validations series of round robin tests with standardized samples as well as real world nanomaterials have been executed.

P1-03

Miniaturized Cartridge for Asymmetrical Flow Field-Flow Fractionation

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Asymmetrical Flow Field-Flow Fractionation (AF4) is a separation technique applicable to particles in a wide size range (1 nm - 100 µm). In current AF4 cartridges, a particle-containing liquid sample is injected into a long (~28 cm), narrow (~1 cm) and very shallow (~350 µm) ribbon-like channel. A cross-flow of solvent, which pushes the particles against a semi-permeable membrane, is applied perpendicularly to the main flow direction. Particles of different size exit the channel at different times and are detected by means of suitable optical detectors.

Despite the many advantages of AF4, its adoption for routine use is limited by the large size of currently available separation cartridges, which results in high runtimes and flow rates, increased reagent consumption and lower operational capacity. All these aspects are important from the point of view of cost-effectiveness and sustainability.

The goal of this project is a miniaturization of the standard AF4-cartridge. The advantages that come along with such a scale-down include simplified handling, reduced costs and a potentially disposable cartridge. Miniaturization also offers a way to improve the performance via shorter elution time and therefore less peak broadening. As an intermediate step towards a plastic based, fully disposable FFF cartridge, a scaled-down metal cartridge with a channel length of about 8 cm was realized. The new cartridge was applied for the separation of gold nanoparticles mixtures (5, 15 and 34 nm). The measurement protocols (application time and intensity of the various flows) were adapted to the new cartridge geometry. A separation efficiency comparable to conventional AF4 cartridges could be maintained, despite a size reduction of about 70%.

This work was supported by the European Commission 7th Framework Programme (project SMART-NANO, NMP4-SE-2012-280779).

P1-04

Investigating determining factors of the solubility of CdSe and SiO_2 nanoparticles under near-body conditions

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Nanomaterials show an ever-rising number of applications in consumer products due to their unique properties. The most outstanding property is their small size, which causes a significantly different behaviour in comparison to the respective bulk materials. Apart from the widely known advantages of nanotechnology, concerns about possible harmful effects on the environment and the human health have risen. Particularly because of their broad application area, contact is inevitable. Risk assessment in aqueous systems is crucial to assess the potential danger to human health since nanomaterials are exposed to a variety of liquids in the human body. The determining factor for their stability in aqueous systems is thereby their solubility. In reference to the exposure of humans to nanomaterials, their solubility should be investigated under body conditions. In our experiment, the solubility of nanoparticles under near-body conditions was investigated by inductively coupled plasma mass spectrometry in combination with filtration methods. As a typical example for nanomaterials, SiO_2 nanoparticles in two sizes and CdSe quantum dots were used. Results showed a clear effect of particle size and pH value on the solubility rates at a temperature of 37 °C. Low pH values increased the solubility of CdSe quatum dots whereas the contrary effect was observed for SiO_2 nanoparticles.

P1-05

Transnational Access at VITO through the FP7 QualityNano Research Infrastructure

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QualityNano aims to establish a pan-European analytical research infrastructure whose purpose is to drive high quality research and testing practices for assessment of the potential risks posed by nanomaterials. This four year EU funded project (Grant Agreement N° INFRA-2010-262163) started in February 2011 and comprises 27 top European analytical & experimental facilities in nanotechnology, medicine and natural sciences.

QualityNano's core aim is the creation of a 'neutral' scientific & technical space in which all stakeholder groups can engage, develop, and share scientific best practice in the field. Initially it will harness resources from across Europe and develop efficient, transparent and effective processes. Thereby it will enable provision of services to its users, and the broader community, all in the context of a best-practice ethos. This will encourage evidence-based dialogue to prosper between all stakeholders. QNano will also pro-actively seek to drive, develop and promote the highest quality research and practices via its Joint Research Activities (JRA), Networking Activities (NA) and provision of Transnational Access (TA) functions, with a global perspective and mode of implementation.

The QNano TA component is dedicated to providing Users from the European nanosafety community access to nanomaterials processing, characterisation and exposure assessment facilities. Access to 15 major European research sites is via a single application and evaluation process. Facilities at VITO (http://www.qualitynano.eu/access/equipment-by-taf/belgium-vito.html) for *in-situ* and *ex-situ* nanoparticle characterisation (material characterisation and imaging, mass spectrometry, and chemical analysis) and nanoparticle exposure assessment (*in vitro* assay, proteomics and transcriptomics platforms, and occupational exposure test chamber) will be highlighted.

P1-06

Novel method for accurate, inline sizing of nano particles

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Nanoparticles are increasingly used in a variety of applications, such as in coatings, paints, sun creams, etc. To optimize the production of these nanoparticles there is a need for in-line quantitative measurements of their size distribution and concentration. Ultrasound-based methods are particularly suitable for particle sizing, as they are non-destructive, non-invasive, fast and relatively cheap. Also, these techniques can be used in opaque dispersions and at high concentrations. However, using ultrasound to measure very small (nano)particles presents a particular challenge for ultrasonic devices, since this requires very high frequencies and precision. In the current work the performance of a novel ultrasonic method is evaluated using SiO2 nanoparticles.

The measurement method is based on ultrasound transmission spectroscopy, where the effect of the particles on ultrasound propagation is measured. The basic equipment for a transmission spectroscopy measurement consists of a transmitting transducer, a liquid medium in which particles are suspended and a receiving transducer. The investigated dispersions consisted of SiO2 nanoparticles (1.38 vol%) dispersed in water. Three batches, provided by Nano-H S.A.S., partner in the In-sight project, had monomodal size distributions with mean sizes 150, 302 and 422 nm. Also two bimodal size distributions were investigated: 1) a mix of 50% 302 nm and 50% 422 nm particles, and 2) a mix of 50% 150 nm and 50% 422 nm particles. As a reference the size distributions were measured using a Malvern device based on optical methods.

The concentrations obtained by the ultrasonic instrument were very similar to the reference concentration (error <10%). The shapes of the particle size distributions were very similar to those of the reference distributions. Moreover, the instrument was even able to produce the correct results for both monomodal and bimodal distributions.

P1-07

Quantifying hard and soft protein coronas around silver nanocubes with localised surface plasmon resonances

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It is known that, upon contact with biological fluids, nanoparticles (NPs) get coated with biomolecules forming a corona. This corona is thought to modulate the interactions with cells.

In the present work we investigate the strongly and weakly binding protein layers around PVP-coated silver nanocubes (AgNCs) in media relevant for nanotoxicology studies. This is done taking advantage of AgNCs specific plasmonic signals. Localised surface plasmon resonance (LSPR) measurements were performed in the UV-Vis region, on NPs incubated in culture medium containing various amounts of foetal bovine serum. Several time-points, from 15 minutes to 24 hours, were studied, showing kinetics of corona formation.

Experimental results of shifts in the wavelength of maximum absorbance were coupled with finite-difference time-domain (FDTD) simulations of the optical response. The resulting model shows the formation of a strongly-binding protein monolayer increasing in density up to the limit imposed by random sequential absorbance. Furthermore, it allowed us to estimate the average number of protein molecules bound to a NP at each time-point. The results at 24 h incubation were confirmed by experimentally quantifying total strongly-bound proteins with the bicinchoninic acid assay. Subsequently, weakly-binding protein layers were studied. We showed soft corona patterns rapidly change upon variation in the serum content. Furthermore, taking into account the thickness of the strongly-binding protein layer and the near field decay, we could quantify weakly-interacting proteins.

The LSPR spectrum of AgNCs exhibits two different, spectrally resolved resonance modes which FDTD simulations show have different spatial distributions and we explore the possibility to distinguish the different corona-formation patterns at cube facets versus cube rounded corners.

The results indicate that LSPR is a sensitive tool for probing the protein corona around nanoparticles *in situ* in biological media.

P1-08

Silver content, silver release and bioactivity of silver-based products

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Consumer products with antimicrobial activity are well known in everyday life. An increasing percentage of these are based on the incorporation of silver species that are able to release silver ions as antimicrobial agent. Many of these products are assumed to contain silver nanoparticles that are estimated to be marketed in annual quantities of around 20 tonnes [1].

We investigated twenty different consumer products and fabrics, including wipes, insoles, band-aid, and shirts, regarding their antimicrobial activity and compared this activity with the total silver content before and after extraction in water and artificial sweat. The antibacterial effectiveness was determined qualitatively, using a recently developed assay, based on the β-galactosidase activity of Escherichia coli. The total silver content was measured by inductively coupled plasma optical emission spectrometry (ICP OES) and graphite furnace atomic absorption spectrometry (GF-AAS). Environmental electron microscopy (ESEM) and energy dispersive X-ray (EDX) measurements were used to identify the silver species present in the products. Silver nanoparticles were identified in one type of band aid also exhibiting antimicrobial activity. We therefore analyzed the dissolution of incorporated silver nanoparticles by correlative transmission electron microscopy (TEM). We found that silver nanoparticles on a thin metallic aluminum foil dissolved due to an electrochemical Ostwald ripening process in oxygen containing artificial sweat.

The results indicate that the antimicrobial activity is not correlated with the total silver content but rather with the amount of silver released into the surrounding.

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P1-09

Bright fluorescent silica nanoparticle probes for high resolution STED and confocal microscopy

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We describe an effective method for the preparation of fluorescent silica nanoparticles in the size range between 30 nm and 130 nm based on l-arginine controlled hydrolysis of TEOS in a biphasic cyclohexane/water system. The obtained particles show a narrow size distribution and are applicable for high resolution STED microscopy. Far-red fluorescent dyes (Atto647N, Dy647, Dy648, Dy649) were either covalently incorporated into the particle matrix or applied as fluorescent layers covered by a silica layer. Covalent attachment to the silica matrix was achieved by aminosilane coupling. For Atto647N, introduction of a cysteic acid spacer was necessary to yield spherical particles with low dispersity.

As revealed by dynamic light scattering and zeta-potential measurements, all particle suspensions were stable in water and biological medium. The spectroscopic characteristics were similar to the behavior of the free uncoupled dyes. Our approach allows a step-by-step formation of multi-colored silica spheres either in the form of dye@SiO2 core-shell architectures or as dye-incorporated spheres. The particles can be used as versatile fluorescent probes in confocal and STED imaging.

P1-10

Synthesis and characterisation of graphene oxide from graphene

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Graphene, a two dimensional monoatomic building block of a carbon allotrope with sp2 hybridized carbon atoms arranged in hexagonal honey comb like structure arose as center of material research of 21st Century. It has received a worldwide attention for its wonderful properties in wide range of applications such as in bio-medical, energy storage, sensor, and optics etc. Graphene possess exceptional properties and shows its promising effects on graphene based devices[1].

In this work, a commercially available graphene grade was employed for investigation and was oxidized via Staudenmaier[2] and Hummers' method[3]. The XRD results show marked difference in the peak patterns of pristine graphene from that of graphene oxide (GO). These differences are due to presence of different functional groups in GO. TEM shows clear exfoliated graphene layers and few transparent single to bi-graphene layers. A comparative study will be reported via FTIR to present difference in functional groups on graphene oxide obtained via both Staudenmaier and Hammers method. All the results were rationalized and are presented briefly.

Keywords: Graphene, Graphene oxide, Hummers' method, Staudenmaier method.

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P1-11

Characterization of gold- and silica nanoparticles with field-flow fractionation and off-line analysis

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Reliable detection and detailed characterization of nanoparticles (NPs) is fundamental for analyzing possible risks and the fate of nanoparticles in the environment [1]. Established methods, for example, electron microscopy or dynamic light scattering, are unsuitable for detecting particles in complex environments, do not separate different particles, and have to be combined to provide sufficient information. Field-flow fractionation (FFF) separates the particles according to their hydrodynamic diameter and can be equipped with multiple sequential detectors [2]. Challenges for FFF on engineered NPs are adsorption and agglomeration of NPs during analysis. They lead to sample loss and biased results. Our goal is to quantify and understand these processes and to develop methods and protocols that avoid them.

Here, we present the results of conventional off-line and multi-detector in-line FFF analysis of freshly synthesized gold and silica nanoparticles with mean diameters between 15 and 105 nm that have narrow size distributions. The particles were characterized using transmission electron microscopy, dynamic light scattering, optical spectroscopy and zeta-potential measurements. FFF measurements were performed using in-line static and dynamic light scattering, UV-VIS transmission measurements and fluorescence spectroscopy. We report relative recoveries of gold NPs and infer different mechanisms for membrane adsorption and NP agglomeration during FFF.

UV-VIS based relative recoveries indicated that less polyethylene glycol (PEG)-modified particles were lost during analysis compared to citrate-stabilized. We also found different loss levels for different membrane materials. In the future, we will expand this systematic study to find optimal materials and protocols for low-loss particle detection and analysis.

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HUMAN HEALTH: *in vitro*, *ex vivo* AND *in vivo* TESTING

P2-01

Effects of colloidal nanoparticles on membrane-dependent signalling in rat lung epithelial cells

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Due to the broad range of specific reactivity, engineered nanoparticles may interact with cells and their physiological reactions. Earlier studies of the group showed that non-colloidal nanoparticles in epithelial cells induce toxic effects in a dose range below the occurrence of acute cytotoxicity by interfering with signalling pathways at the level of the membrane receptor EGFR.

The present study aimed to investigate whether similar effects can be induced by colloidal nanoparticles. For that purpose nanoparticles consisting of silica, gold, silver, and magnetite (SiNP, GNP, SNP and MNP) were tested for their ability to interfere with signalling pathways in rat lung epithelial cells (RLE-6TN). Nanoparticles were characterized physically and chemically by scanning electron microscopy, dynamic light scattering and photometry.

Initial studies employing neutral red, trypan blue and WST assays identified doses which did not induce cytotoxicity in RLE cells up to 24 h of exposure. Oxidative stress which may influence signalling pathways was investigated in cell-free systems or intracellularly using the fluorescent dye 2.7 dichlorodihydrofluorescein diacetate. None of the chosen doses significantly elicited reactive oxygen species. Accordingly, none of the exposure scenarios was able to trigger the activating phosphorylation of EGFR. However, exposure of lung epithelial cells with GNP and SNP showed a significant reduction of the EGF-induced phosphorylation of EGFR (Y1173) after 24 h. As a consequence of this specific nanoparticle cell interaction, proliferation of RLE cells in the presence of EGF was significantly and dose dependently reduced in experiments up to 72 h.

The data for the first time demonstrate an interference of colloidal nanoparticle with receptor mediated cell proliferation which is contradictory to the well known activating effect of non-colloidal nanoparticles.

P2-02

Protein corona of metallic nanoparticles and their interactions with human macrophages of the peripheral blood

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Metallic nanoparticles are widely used in cosmetics and medical treatment. However, information about their interaction with the immunosystem are scarce. In this work, the interaction of macrophages isolated from peripheral blood mononuclear cellls (PBMC) in contact with titanium dioxide nanoparticles, iron (III) oxide nanoparticles and iron (II, III) oxide nanoparticles are described. Characterization of the nanoparticles protein corona followed by evaluation of cytokines production after interaction of nanoparticles with cells were done. SDS-PAGE electrophoresis and a subsequent mass spectroscopy analysis to separate and identify proteins from corona, respectively, were performed. Immunomodulatory effects of nanoparticles were analyzed determining the concentration of pro- and anti-inflammatory cytokines in supernatants of cell cultures using enzyme-linked immunosorbent assay (ELISA). An increase in interleukins 6 concentrations during the first three hours incubation was observed followed by a time-dependent decrease. This decrease in interleukins 6 concentration presented relation to the time-dependent cytotoxicity on the cells. Information about the protein corona of nanoparticles can be useful to correlate the immunological responses of blood macrophages to metal nanoparticles.

P2-03

Uptake kinetics of polystyrene nanoparticles in CD34+ hematopoietic stem cells and CD34-derived dendritic cells

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The dramatic rise in the number of nanotechnology-based products implies increased human exposure and consequently, potential health impacts have to be considered. Derangement of the developing immune system is one such health effect of concern, as it can lead to immune suppression or, more importantly, to increases in the incidence or severity of allergic and autoimmune diseases. To be able to study the potential impact of synthetic nanoparticles (NPs) on immune system development, it is necessary to understand whether and how NPs are taken up by relevant cell types. In the present study, NP uptake by human cord blood-derived CD34+ hematopoietic stem cells (HSC) was compared to myeloid-type dendritic cells (CD34-DCs), which were derived from CD34+ HSC through subsequent steps of proliferation and differentiation. Cells were exposed to well-defined, 40-nm size fluorescently dyed carboxylated polystyrene NPs. NP uptake was assessed by means of flow cytometry and time kinetics were evaluated by monitoring the uptake at different time points up to 24 hours. Both HSC and CD34-DCs promptly accumulated NPs within the first hour after exposure. NP uptake HSC stagnated within the initial hours. In contrast, CD34-DCs continued to increase the intracellular amount of NPs up to 24 hours post-exposure. The settlement of the equilibrium can be the result of an arrest in NP uptake, or due to continued uptake in combination with export. Changes which occur after proliferation and differentiation of HSC towards CD34-DCs might be responsible for the divergence of the responses of both cell types suggesting, different uptake mechanisms and/or intracellular fate. In order to elucidate the involved cellular pathways, future experiments will be performed to investigate the uptake mechanisms and fate of the NPs by flow cytometry and fluorescence microscopy.

P2-04

Influence of gold nanoparticles on wound healing *in vitro*

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Nanomedicine is an emerging field expanding rapidly because of the development and incorporation of new nanocomposites into a range of product and technologies. Previous studies showed that gold nanoparticles could be applied therapeutically for intravascular and percutaneous drug, gene therapy and also wound healing process (1).

In our experiment we used gold nanoparticles tested on *in vitro* wound healing model prepared from human fibroblasts. The tested concentrations (25ppm, 2.5ppm, 0.25ppm) were chosen from MTT test. The production of inflammatory cytokines (IL-6, IL-8, IL-12 and TNF-α) and VEGF, bFGF, GM-CSF were evaluated using multiplex bead-based assay. The effect of gold nanoparticles on production of MMP-2 and MMP-9 was also detected.

The gold nanoparticles decreased the production of TNF-α, GM-CSF and IL-12 after 48 hours and also decreased production of VEGF for all tested concentration after 6, 24 and 48 hours. The production of IL-8 was increased after 24 and 48 hours.

Acknowledgment: This work was financially supported by the FR-TI2/205 and LF_2013_008.

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P2-05

Nanoparticles in the gut are trapped by intestinal mucus

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The omnipresence of nanoparticles (NPs) in many goods has lead to a constant risk of exposure and inadvertent uptake for humans. This calls for a thorough investigation of the consequences of NP intake. As the vast mucosa of the human intestinal tract represents an attractive site of entry we wanted to take a look on the fate which ingested NPs suffer in the gut. As a model to investigate NP uptake we used the isolated perfused rat small intestine. Differently sized fluorescent latex particles were used as exemplary NPs. The particles were administered as bolus into the isolated intestine, and samples from the luminal, vascular and lymphatic compartments were collected over the time. NP amounts in the different fluids were determined by fluorescence measurements.

No particles could be detected in the vascular and lymphatic samples, only in luminal samples a major amount of NPs was found. Yet, a substantial fraction of NPs could not be recovered in the fluid fractions. A histological examination revealed that virtually no particles adhered to the epithelium or resided in the tissue, the bulk of NPs seemed to be trapped in the mucus lining the gut tube. When this mucus was dissolved and collected almost the entire amount of particles missing could be recovered: over 95% of the given NPs were present in the two fractions, luminal and dissolved mucus. To promote NP uptake by an extended interaction with the epithelium, the peristalsis was decelerated and the duration of the experiment was prolonged. Even under those conditions, no particle fluorescence was detected in the vascular and lymphatic samples. In conclusion we could show that after intestinal exposure with a large dose of NPs the vast majority of NPs did not come into contact with the epithelium but was either directly discarded from the gut or trapped in mucus. The healthy intestinal tract obviously provides an effective barrier against NP uptake whereby the mucus film seems to play an important role.

P2-06

Anti-inflammatory responses of lipopolysaccharide-induced human skin fibroblasts upon exposure to gold nanoparticles

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Biomedical applications of gold nanoparticles (AuNPs) are rapidly increasing due to their excellent properties of relatively low cytotoxicity, a high capacity to target cells and easily functionalized surfaces. Gold compounds have long been used to treat some inflammatory diseases including rheumatoid and psoriatic arthritis [1]. Currently, AuNPs are used effectively either as a diagnostic imaging agent or as a therapeutic agent in experimental gene and drug delivery [2]. With the increasing interest in the use of AuNPs in medical applications grow at the same time concerns about the potential toxicity risk of AuNPs. But the question of the toxicity is quite controversial [3]. Numerous studies report no adverse biological effect in either *in vitro* or *in vivo* experiments but there are some reports exerting a measurable influence on some aspects of cellular activity including cytotoxicity, down-/up-regulate inflammatory, suppress cell proliferation, bind to or interfere with DNA etc. [4]. The present study investigates the effect of AuNPs (colloidal gold solution) on lipopolysaccharide-induced inflammation in normal human dermal fibroblasts (NHDF).

NHDF (1x105 cells/cm2) were pre-treated with lipopolysacharide (10 μg/ml; 8 h) and then the AuNPs (0.25, 2.5 and 25 ppm) in serum-free medium were applied for 12/24 h. The production of cytokines (IL-6, IL-8, TNF-, IL-10 and IL-12) were monitored by Bio-plex suspension array system. The expression of COX-2 and IL-6 was evaluated by western blot analysis.

The different effect of gold nanoparticles on the selected parameters in NHDF will be discussed.

Acknowledgement:

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P2-07

Effect of silver nanoparticles on stem cell differentiation and immune reaction *in vitro*

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The need for risk assessment and new methods to appraise the humantoxicity and immunosafety of nanomaterials is becoming urgent with increasing use of nanoparticles (NPs) in several areas of life. Due to their antimicrobial properties, silver NPs are used in a huge number of consumer or medical products. However, there is a serious lack of information concerning the biological activity of nanosized silver in human tissue cells. An influence of NPs on human health might lead to unforeseen consequences to the immune system and could cause diseases as cancer. Negative effects of NPs on stem cells might result in inaccurate tissue function.

Therefore the effect of silver NPs on stem cell differentiation and the induction of an inflammatory response were investigated in this study.

The effects of silver NPs (10 nm and 80 nm) on adipogenic differentiation of human bone marrow mesenchymal stem cells were investigated regarding lipid vacuole formation and mitochondrial activity at different time points (chronically exposure time 21 days). Further the silver NP-induced inflammatory response was non-invasively quantified using stably GFP-transfected reporter cells.

Via time lapse microscopy the differentiation progress as well as the silver nanoparticle-induced inflammatory response were continuously investigated under chronically silver NP treatment.

In this preliminary study, chronically treatment of adipogenic differentiating hMSCs with silver NPs resulted in a reduced number and size of lipid vacuoles and reduced mitochondrial activity depending on the applied concentration and the surface charge of the particles. Further a dose-dependent inflammatory response was triggered by the silver NPs.

The present study clearly shows that nanosilver might adversely affects human health; so there is a need for risk assessment and regulations for the use of nanomaterials in consumer products.

P2-08

Effect of manufactured gold nanoparticles on allergen-induced sensitization

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The adjuvant activity of air pollution particles on allergic airway sensitization is well known, but a similar role of manufactured nanoparticles in allergic sensitization has not been clarified. The goal of our study was to assess the possible effect of manufactured nanoparticles (NPs) on an allergen-induced sensitization response.

Immature myeloid dendritic cells (CD34-DC) that were *in vitro* differentiated from human cord blood-derived CD34+ progenitor cells were exposed to sensitizing agents and spherical gold NPs (50 nm) for 24 hours, either as separate inducers or as a mixture. The allergens that were used are the sensitizing metal nickel sulphate (NiSO4) and the Der p 1 protein, a major house dust mite allergen. The sensitization response was assessed by looking at CD34-DC maturation by measuring cell surface expression of HLA-DR, CD11c and the co-stimulatory molecules CD80, CD86 and CD83 using flow cytometry.

Exposure of CD34-DC to gold NPs induced upregulation of the co-stimulatory molecules. Der p 1 on its own did not induce maturation and the response obtained after co-exposure with the NPs was comparable to sole NP exposure. Conversely, upregulation of co-stimulatory molecules in CD34-DC was observed after sole exposure to NiSO4. When NPs were added simultaneously with the metal ions, this maturation response was significantly inhibited. We hypothesize that this may be caused by a physical interaction between the gold NPs and nickel(II) ions, as previously described for other metal ions, but this needs to be clarified further. Insights in the possible modulating effect of gold NPs in allergenic responses, as presented in this study, will further promote safe and efficacious design and use of nanoparticles in consumer products and technological applications.

P2-09

Molecular mechanisms of EGFR activation by carbon nanoparticles in lung epithelial cells

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Background: Particulate air pollution induces pathogenic endpoints like proliferation, apoptosis, and pro-inflammatory reactions in lung epithelial cells. The activation of the epidermal growth factor receptor (EGFR) is a key step responsible for signaling events specific for these endpoints. Earlier experiments identified particle-derived reactive oxygen species (ROS) as one possible trigger eliciting the observed EGFR activation. As initial molecular mechanism responsible for the activation of this signaling event, we hypothesize a ligand-independent internalization of EGFR involving caveolin-1.

Methods: Membrane signaling events were studied in isolated lipid rafts from lung epithelial cells treated with environmental model particles (carbon nanoparticles) with regard to lipid and protein content of the signaling platforms. Using positive and negative intervention approaches, lipid raft changes, subsequent signaling events, sphingomyelinase activity, and lung inflammation were investigated *in vitro* in lung epithelial cells (RLE-6TN) and in vivo in exposed animals.

Results: As a typical pattern of cell reaction after exposure to carbon nanoparticles, EGFR activation and internalization were observed. First results indicate the involvement of caveolin-1 as a marker of ligand-indepedent activation of the receptor. Further analyses demonstrate that carbon nanoparticles triggered intracellular oxidative stress is responsible for an increase of ceramides in lipid raft structures and the subsequent EGFR signaling. In this context, the activity of neutral sphingomyelinase (nSmase) was determined and interestingly, carbon particles rather inhibited the enzyme activity, indicating a non-enzymatic generation of ceramide.

Conclusion: The data identify the ligand-independent, mechanism of EGFR activation as a result of the accumulation of ceramides in lipid rafts of lung epithelial cells as initial molecular event contributing to the toxicity of inhaled carbon particles.

P2-10

Cytotoxicity, oxidative stress, and genotoxicity induced by molybdenum nanoparticles in mouse skin fibroblast (L929) cells

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Molybdenum nanoparticles (Mo-NPs) have wide application and, are being used as stabilizers in alloys for hi-tech applications, cutting tools, textiles, microelectronics films, coatings, plastics, nanowire, and X-ray tubes. However, biological or cellular responses to Molybdenum nanoparticles are not been explored so far. Therefore, the present investigations were aimed to develop an *In vitro* model system to assess the cytotoxicity, oxidative stress, and genotoxicity induced by Mo-NPs using mouse skin fibroblast (L929) cells. Cells were exposed to various concentrations (1 to 100 □g/ml) of Mo-NPs for 24 and 48 h. After the exposure, cytotoxicity by MTT and NRU assays, and oxidative stress by LPO, GSH, and catalase assays were studied. Further, intracellular ROS generation, mitochondrial membrane potential, cell cycle arrest, and DNA damage by Comet assay were studied following the exposure of Mo-NPs. MTT and NRU assays revealed a concentration dependent decrease in the cell viability of L929 cells. These cytotoxic responses were in concurrence with the markers associated with oxidative stress such as, an increase in lipid peroxidation (LPO), and a decrease in the levels of glutathione (GSH) and catalase activities. Further, an increase in ROS generation and decrease in the mitochondrial membrane potential were observed. The cell cycle analysis and Comet assay data revealed the G2/M arrest and DNA damage respectively induced by Mo-NPs in L929 cells. Data of this study indicate that Mo-NPs induced cytotoxicity, oxidative stress and genotoxicity in L929 cells.

P2-11

The magnitude of lung inflammation induced by carbon nanoparticles is connected to the internal circadian rhythm of the lung

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Throughout the daily rhythm, the lung shows physiological and functional changes. Disruption of the circadian rhythm is associated with alterations in the immune system and its dysfunction as seen in chronic lung diseases. The 24-hour rhythm is regulated by the central clock in the brain. However, circadian timers (Zeitgeber) are also found in peripheral tissues. Physiological changes of peripheral organs due to a malfunction in its circadian rhythm may be an important factor of pre-disposition causing significant differences in the response to xenobiotics.

To investigate a time-dependent sensitivity to combustion-derived inhalable particles, carbon nanoparticles were applied to C57BL/6 mice at four different time points throughout the day. Twelve hours after instillation, inflammatory parameters were analyzed in the bronchoalveolar lavage (BAL). Significant differences in neutrophilic lung inflammation were observed depending on the time point of particle application. Additional dose response experiments demonstrate dramatic differences in sensitivity to various quantities of carbon nanoparticles (CNP) during the daily time course. The relevance of the lung internal rhythm was investigated by an inverted feeding experiment. Therefore, the circadian rhythm of peripheral organs was uncoupled from the light stimulus by feeding the mice at the light phase or at night. The previously observed sensitivity to instillation was abrogated when interfering with the feeding rhythm. Reciprocally, the pulmonary circadian rhythm (per2) was shown to be disrupted after CNP exposure.

We see strong differences in lung inflammation depending on the time point of exposure. The inflammatory response to inhaled particles was affected by a change in feeding times. The data indicate that CNP exposure interferes with the organ-specific circadian rhythm of the lung. Still, the underlying circadian mechanisms regulating the neutrophil-dominated acute inflammation remain elusive.

P2-12

Differences in effects of similar sized Ag NPs on an intestinal co-culture model: testing considerations and concerns

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The aim of the study was to elucidate the observed differences in effects of similarly sized Ag NPs (23, 24 and 27 nm) synthesized in a biological way on an intestinal co-culture model (Caco-2/TC7: HT29-MTX cells).

Effects of Ag NPs and AgNO3 on metabolic activity, viability and monolayer integrity were assessed (alamar blue, MTS, LDH and transepithelial electrical resistance (TEER) measurement). Ag uptake was evaluated with NanoSIMS50 and the ion dissolution was assessed with ultrafiltration and ICP-MS.

AgNO3 and all Ag NPs induced a dose-dependent decrease in metabolic activity (alamar blue assay) of the un-differentiated cells in co-culture with Ag 23 nm being the most potent followed by Ag 24 and 27 nm. This decrease in metabolic activity was attenuated by the addition of N-acetyl cysteine (NAC) to AgNO3 and Ag 23 nm suspensions. At a concentration of 1 mg/L Ag 23 nm and Ag 24 nm similar levels of Ag were released (2.5 µg/L after 6 hours of exposure) followed by Ag 27 nm.

Ag 23 nm and AgNO3 led to a decrease in monolayer integrity of differentiated cells in co-culture in a dose-dependent manner which corresponds to an increase in the amount of Ag found in the basolateral compartment, suggesting an effect on the barrier function. A decrease was already observed at concentrations that had no effect in the metabolic activity assays. Therefore, that could be an important additional endpoint. According to NanoSIMS50 analysis an uptake of Ag was observed for all NPs and AgNO3 that seems to be homogenously distributed.

Our study highlights the importance of considering suitable assays for Ag NP toxicity testing and that NP size or ion dissolution alone cannot predict and explain differences in effects observed for similar sized Ag NPs.

P2-13

Biopolymer nanoparticles for therapeutic applications: Synthesis, characterization and assessment of biocompatibility

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Biodegradable nanoparticles (NPs) as drug delivery systems (DDS) become extremely attractive for medical applications, due to their stability in the blood stream and controlled release characteristics.

In this study, fluorescence-labeled polymer-based NPs were synthesized and characterized according to their physiochemical properties, followed by the biological evaluation as potential therapeutic DDS.

Poly(lactide-co-glycolide) (PLGA) NPs were prepared using a solvent-displacement or emulsion-evaporation method. The PEGylation degree was varied using mixtures of PLGA and a PLGA-poly(ethylene glycol) (PEG) diblock copolymer as starting material. The preparation process was optimized to achieve monodisperse NPs at particle diameters of 100 and 200 nm. By centrifugal field-flow fractionation technology time resolved-NP sizes were quantified, which varied upon the solvent. *In-vitro* investigations of the cellular binding, the mechanisms of the NP-cell-interaction, the pro-inflammatory cell response and the toxicological potential were performed in different human epithelial cell lines, GFP-transfected reporter cells and macrophages.At all three tested time points (1, 4, 24 h) the exposure with PEG-PLGA NPs (0–100 µg/ml) resulted in a time- and dose-dependent cellular binding and NP-uptake. Further the increases in PEGylation decreased the cellular binding. On the other hand, PEG-PLGA NPs did not affect the cell metabolism and proliferation and did not trigger pro-inflammatory reactions and cell death. The growth inhibition value confirmed the biocompatibility of the NPs.In first studies PLGA-NPs loaded with an anticancer reagent comprise no cytotoxicity as shown in primary cell cultures of macrophages.The present study clearly shows the biocompatibility of the homogeneously synthesized PEG-PLGA NPs. In particular, the ability for particle targeting and drug loading gives them specific therapeutic properties and makes them a promising candidate for new biodegradable DDS.

P2-14

Are precision-cut lung slices (PCLS) an effecive tool for *in vitro* nanotociology studies?

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Background: Precision-cut lung slices (PCLS) are an established *in vitro* alternative to in vivo experiments in pharmacotoxicology. The aim of this study was to evaluate the potential of PCLS as a tool in nanotoxicology studies. Here, we used silver nanoparticles (Ag-NP) because these particles have been previously shown to induce a cytotoxic and inflammatory response in several in vitro and in vivo studies.

Methods: 250-µm thin rat PCLS were exposed to three concentrations (10, 20, and 30 µg/ml) of 70-nm monodisperse PVP-coated Ag-NP under submerged culture conditions *in vitro*. 1.7-µm quartz particles served as 'non-soluble' and 200-nm zinc oxide (ZnO-)NP as 'soluble' control particles. After 4 and 24 h, cell viability was measured with the WST-1 assay. Furthermore, the release of the inflammatory cytokines TNF-α and CXCL1 was determined using ELISA. In addition, multiphoton microscopy was used to assess the localization of Ag-NP in PCLS after 24 h of incubation.

Results: Incubation of PCLS with quartz particles and Ag-NP at all concentrations did not induce a significant loss in cell viability. After 4 and 24 h of incubation, only ZnO-NP decreased cell viability to 47% and 0%, respectively. Interestingly, none of the particles tested induced an inflammatory response in PCLS. Finally, multiphoton microscopy revealed that the Ag-NP were localized predominantly at the cut surface but not inside PCLS.

Conclusions: Exposure of PCLS to Ag-NP or 'non-soluble' quartz particles for 4 or 24 h did not result in WST-1 conversion or an inflammatory response, while incubation with 'soluble' ZnO-NP for 24 h caused complete cell death. Moreover, the localization of NP predominantly at the cut surface of PCLS indicates that 'non-soluble' nanoparticles will only interact with potentially damaged cells at the cut surface rather than be translocated to inner tissue regions. In conclusion, our findings suggest that PCLS are of only limited use in nanotoxicology studies.

P2-15

Uptake and intracellular distribution of core-shell iron oxide particles in Caco-2 cells

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Superparamagnetic iron oxide nanoparticles (SPION) bear a great potential in medical diagnostics. By combination of material characteristics of contrast agents for magnetic resonance imaging (MRI) and computed tomography (CT), novel contrast agents for dual MRT/CT imaging of the small and large intestine should be put into practice. For a safe application adverse effects on the human health have to be excluded by proper testing.

In this study we investigated the uptake, intracellular distribution and cytotoxicity of core-shell iron oxide nanoparticles in Caco-2 cells, representing a cell culture model for the human intestine. Cells differentiated on cell culture inserts (ThinCerts) were incubated for up to 72h with fluorescently labelled nanoparticles using concentrations of 0,2mM or 2mM Fe. Uptake and distribution were visualized using confocal laser scanning microscopy (CLSM) and electron microscopy (EM).

Incubation of Caco-2 cells with nanoparticles induced no changes in cell morphology and no cytotoxicity could be observed using up to 2mM Fe. Particles became internalized within less than 2h incubation time. In EM micrographs particle aggregation could be observed and particles stick on the microvilli of the cells. In further studies by staining different cell compartments like the mitochondria the interaction of nanoparticles with these compartments will be investigated.

The work was funded by the German Federal Ministry of Education and Research (BMBF), in frame of the project “NanoKon”, FKZ 03X0100C.

ENVIRONMENTAL IMPACT OF NANOMATERIALS

P3-01

The effects of nano-TiO_2 particles on bacteria and algal cell inactivation and lipid oxidation

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The nanotoxic effects of metal oxide nanoparticles (MO-NPs) are still poorly documented while their commercialization increases in many production and manufacturing sectors. Among the various MO-NPs, TiO2 has being the most used NPs in industry; from wall paints to cosmetic products and from textile products to children toys. Besides their advantages and they are regarded as a biocompatible material in the absence of photoactivation, metal oxide NPs have shown to exhibit strong cytotoxicity when exposed to UV and solar irradiation.

The potential eco-toxicity of nanosized TiO_2 suspensions was investigated using E. coli bacteria and P. subcapitata algae as test organisms. These photosensitive nanomaterials were found to be harmful to varying degrees, with antibacterial activity increasing with primary particle sizes from 16 nm to 20 nm. The presence of light was a significant factor under most conditions tested, presumably due to its role in promoting generation of reactive oxygen species (ROS). ROS oxidize lipids of organic matter, in this case the lipids in bacterial cell membrane and algae cells. There have been studies that show TiO2 can inhibit growth of algae and bacteria. Moreover, bacterial die-off was observed under dark conditions, indicating that undetermined mechanisms additional to photocatalytic ROS production were responsible for toxicity. The dose-response experiments were run to determine the EC50. For *E. coli* and *P. subcapitata* the approximate EC50 values are >150, 300 mg/L, respectively. Cell membrane damage was observed in terms of lipid peroxidation (e.g., production of malondialdehyde, MDA), Confocal microscopy and Scanning electron microscopy (SEM) were employed to examine the organisms. The findings show that nanoparticles are affecting the organisms studied. Continued research in needed to determine how the particles are interacting with each organism and whether this will have an impact on how each use and dispose of these particles.

P3-02

SIRENA PROJECT: SImulation of the RElease of NAnomaterials from consumer products for environmental exposure assessment

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In a previous FP7 founded project (Nephh GA N 228536), it was demonstrated that physical processing at different stages of the life cycle of nanocomposites conveys the release of Engineered Nanomaterials (ENMs).

The objective of Sirena Life project is to demonstrate and validate a methodology to simulate this unintended release of ENMs from consumer products by replicating different life cycle scenarios. Adopting it, nanocomposites industry will get information for evaluating the risk of embedded ENMs release and associated exposure. Three relevant industrial sectors (aeronautical, automotive and energy) and two ways to physically process samples (drilling and crashing) simulating different life cycle scenarios have been selected.

During the first ten months of project (January- October 2013), a Technological Surveillance System (TSS) has been designed and implemented in order to properly trace and assess relevant information in the areas of interest of Sirena.

The different combinations of nanofillers for each polymeric matrix representative of the three industrial sectors (epoxy, polypropylene and polyester) have been defined taking into account the properties of the material to be improved. Samples are being produced and their properties verified. Once the improvement in the functionality that these ENMs confer is verified, the (nano)release-simulation processes will be carried out.

Expected results from Sirena include:

-A searchable database with outcomes from the TSS

-A state of the art report: methods to simulate the release of NMs from consumer products

-Evaluation of exposure scenarios

-Exposure data

-Methodologies and prototypes for Environmental Scenario Replication

-Best practices manuals

The project (January 2013-December 2015) is co-financed by the Life+ Environment Policy and Governance (Life11 ENV/ES/596). The Coordinating Beneficiary is Inkoa Sistemas and Tecnalia R&I and Cranfield University are Associated Beneficiaries.

P3-03

Galvanic manufacturing as a source of metal nanoparticles

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The development of new galvanic technology is important for social-economic growth. Galvanic manufacturing is widely represented nearly in every average populated Russian city. Under the environmental protection act in Russia, the regulations for galvanic manufacture (GM) set at the end of the last century are not included in the regulations and safety standards for ambient ultrafine and nanosized particulate matter (PM). The hazard posed by galvanic productions to urban environment is an emerging concern that recently got a lot of public attention. Nanosized metals (NMe) may present a variety of hazards for environmental and human health. Therefore, keeping the fabrication cost and environmental foot-print to a minimum is critical. To address this issue, we tested levels of NMe PM in snow samples collected around GM in two Far East Russian cities (Ussuriisk, Blagoveshchensk). The samples were collected from clean recreational park areas and from urban area close to GM sites within 200-500 m distance. Employing transmission electron microscopy, energy-dispersive X-ray spectroscopy, ICP-MS, and laser diffraction particle size analyzer, we found accumulation of high level of Fe, Cr and Fe/Cr-alloy Me NPs with size distribution ranging 10-120 nm present in industrial sites compared to the controls collected from the clean recreational areas. Here we are for the first time reporting that Me NP of Pb, Al, Cr, Fe. Ni, Cu, and Zn were detected around galvanic shop settings. Therefore, surveillance of environmental and occupational exposure and risk assessment in galvanic industry workers are warranted.

The work is supported by the Scientific Fund of the Far Eastern Federal University and Presidential Grant for supporting young scientists (MK-1547.2013.5).

P3-04

In vitro screening test and understanding the mechanism on the ecotoxicity: Adverse effect of surface modified silver nanoparticles on luminescent bacteria

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Silver nanoparticles (AgNPs) have been widely used in various fields, including textile, medicine, cosmetic industry, and etc. due to their antibacterial effect. Many recent researches show that surface modified AgNPs and their applications have been extensively studied due to their high biocompatibility, stability, solubility, and targeting potential. As a result of wide and various applications, a possibility of their discharging into the environment, especially for the aquatic environment, would also be increased. Therefore, research on the fate and toxicological evaluation after exposure to the aquatic environment are required.

In this study, acute toxicity screening test of surface modified AgNPs with different capping agents of citrate, tannic acid, BPEI (branched polyethyleneimine), and PEG (polyethylene glycol) were conducted using marine luminescent bacteria such as *Photobacterium leiognathi*, *Vibrio fischeri*, *Vibrio harveyi*, and etc. Effects of physical characterizations of target nanoparticles on the toxicity were compared by EC50 achieved from dose-response curves. According to salt stability and surface charge of target AgNPs, the degree of their toxicity showed in the following order: BPEI-AgNPs (moderate salt stability, highly positive charge) > PEG-AgNPs (high salt stability, moderately negative charge) > Tannic-AgNPs (moderate salt stability, highly negative charge) > Citrate-AgNPs (low salt stability, highly negative charge) with the average EC50 values from 9 marine luminescent bacteria of 2.04±1.41, 4.49±3.79, 4.81±2.43, and 7.08±2.69 mg/L, respectively. In addition of salt stability and surface charge, silver ions released from AgNPs may have an important role on the toxicity, but silver ions from most of target AgNPs were lower than the detection limit of ISE. Therefore, instead of silver ions released from AgNPs, capping agents should be considered as one of factors caused the toxicity of surface modified nanoparticles.

P3-05

Graphene oxide and its antibacterial activity against gram-positive *Staphylococcus spp.*

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Graphene has been fascinating the scientific community with its exceptional properties in wide range of applications for both medical and non-medical fields. Due to these properties, Graphene is known to replace any type of material present in the world. Researchers are now a days tuning graphene either by oxidizing it to obtain some reactive functional groups or by intercalating with intercalating agents to tune it with desired properties within the scope of requirement.

In this work, we employs graphene oxide (GO) obtained through Hummers' method1 to investigate its antibacterial activity against Staphylococcus spp. The graphene oxide was characterized by SEM to study its surface morphology. A clear exfoliation of platelets into transparent graphene layers was seen. FTIR spectra results show the oxygenated functional groups presents were mainly from carboxylic and epoxide family. GO as biological application shows stronger antibacterial activity against bacterial species. Antibacterial activity was tested on different species of *Staphylococcus spp*. gram positive bacteria and will be presented comprehensively.

Key words: Graphene oxide, Hummers' method, *Staphylococcus spp.*

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P3-06

Synthesis, characterization and efficiency studies of different nanoadsorbents for removal of fluoride and arsenate ions from aqueous media

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Fluoride and arsenic are persistent and non-biodegradable pollutants that accumulate in soil, plants, wildlife and in water systems. Therefore, knowledge of their removal, using best technique with optimum efficiency is required. The present survey highlights on efficacy of nanoscaled activated alumina, calcium oxide-modified activated alumina, lanthanum –impregnated alumina and lanthanum –impregnated calcium oxide-modified activated alumina. As a medium having higher sorption capacity than activated alumina, lanthanum oxide has higher isoelectric point (IEP) of 11.1 than activated alumina (9.2). An attempt has been made to study the adsorption process on various key factors (pH, agitation time, initial concentration, temperature, particle size, surface area, presence and nature of counter ions and solvent dose) and the removal capacity of characterized materials are reviewed. A wet impregnation method was used to incorporate lanthanum ions on the surface functional groups. Their adsorption isotherm results showed that the adsorption of each anion followed the Langmuir isotherm without interferences of other anions such as Cl-, Br-, I- , NO3 and SO42-. The results indicate that the La(III) –impregnated calcium oxide-modified activated alumina has shown excellent results for the defluoridation and dearsenification in aqueous media.

DETECTION AND QUANTIFICATION OF NANOOBJECTS IN LIVING SYSTEMS

P4-01

THG microscopy for high-resolution *in vivo* imaging of nanomaterials

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The *in vivo* imaging of the localization and behavior of nanomaterials in cells or tissues is largely restricted to fluorescence microscopy that offers high spatial resolution and signal-to-noise ratio. However, this requires either inherently fluorescent nanomaterials, e.g., quantum dots (QDs), or the attachment of fluorescent labels that, in turn, might alter the properties of the nanomaterial. To address this problem, we assessed the potential of Third Harmonic Generation (THG) microscopy for in vivo imaging of non-fluorescent nanomaterials.

THG microscopy is based on optical effects induced by specific inherent physical properties of a specimen. Recently, we demonstrated that THG microscopy allows high-resolution label-free 4D visualization of cellular and tissue structures, in intact muscle of live mice.

Initially, we defined the multi-photon settings necessary to induce signals from anatase TiO_2 (NM-101) and Ag nanoparticles (NM-300) as well as from carboxyl QDs (Invitrogen) as fluorescent control particles and characterized the spatial resolution of the microscope system under the imaging conditions used (0.9 µm in xy; excitation light 1275 nm, detection 417-477 nm).

Performing THG microscopy of live RAW264.7 macrophages 2 h upon incubation with TiO_2 nanoparticles clearly revealed their localization in intracellular vesicles as well as dynamic movement of these vesicles. By using THG microscopy on skeletal muscle tissue of live mice, we were able to detect TiO_2 as well as Ag nanoparticles in the blood stream immediately after systemic injection. Ag nanoparticles were found to form stable associations with microvessel walls. Additionally, THG provided exact information about the localization of carboxyl-QDs translocated to skeletal muscle tissue 1 h after systemic administration, as verified by fluorescence microscopy.

Taken together, THG microscopy appears to be a suitable tool for high-resolution 4D imaging of nanomaterials in vivo.

P4-02

Use of surface plasmon resonance technique for the specific detection of single biological nano-objects

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Modified surface plasmon resonance imaging (SPRi) technique was reported to be a novel method for the detection of the binding of single nano-particles to sensor's surface [1, 2]. However, bio-analytical features of this SPRi method and specificity of performed detection require further examination. In current study, we demonstrated that modified SPRi technique allows detection and visualization of single inactivated influenza viral particles and HIV-VLPs. The detection was performed in buffers without serum as well as in buffers containing different concentrations of serum (up to 50%). We also showed specificity the binding of biological nano-particles to the functionalized sensor's surface. Furthermore, we investigated the dependence of particle binding rate on the density of antibodies onto the biosensor surface and demonstrated the applicability of modified SPRi technique for the determination of particle concentrations in buffers. During this study we also developed new algorithms and software for the data processing and analysis. Together, our findings open new horizon for SPRi technique in such research areas as viral biology and biology of extracellular vesicles (exosomes and microvesicles).

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P4-03

Using STED-microscopy and image processing to investigate the uptake of silica nanoparticles in lung epithelial cells

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In case of exposure to nanoparticle the lung is one of the main target organs. The interactions of nanoparticles with lung cells are determined by the physicochemical particle properties. Knowledge about nanoparticle (NP) uptake and intra-cellular fate is of great interest for the biomedical as well as toxicological field. As both, nanoparticles and cellular structures are in the nanometer size-range, high-resolution microscopy techniques are needed to study particle-cell interactions *in vitro*.

Therefore, we investigated the interaction of silica nanoparticles with A549 cells as model for human type II alveolar epithelial cells (A549) using stimulated emission depletion (STED) microscopy. The uptake of two different sized fluorescently labeled silica particles (25nm and 85nm) was compared using the same particle concentration (9,2Exp10/ml) after 5h of incubation. Additionally the cell membrane was labeled to distinguish between intra-cellular and external particles. 3D-stacks of whole cells were imaged with a Leica-SP5-STED microscope. Deconvolution, segmentation and quantification of the data was performed with the “object-analyzer”-tool from the “Huygens” software (SVI, Hilversum, Netherlands).

The cells showed no morphological changes, although NPs of both sizes were found inside the cells. In our study in the case of exposure with 85nm particles approximately twice as much objects were found within the cells compared to exposure with 25nm particles.

We recently established dual color STED as a tool for colocalization analysis to get further insights into intra-cellular localization and pathway of NPs in lung epithelial cells.

MODELLING AND PREDICTION OF NANOMATERIAL EFFECTS

P5-01

Collection of toxicity, physicochemical and characterisation data to enable modelling of nanomaterial effects

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A number of EU projects have been established to address concerns about the potential health risks posed by nanomaterials. The NanoPUZZLES project is developing new computational methods for predicting the toxicity of nanomaterials based on Quantitative Structure-Activity Relationships (QSARs), chemical category formation and read-across approaches. Successful application of these approaches requires sufficient quantities of high quality toxicological and physicochemical data on well-characterised nanomaterials to be organised self-consistently within an electronic database. NanoPUZZLES is contributing to the development of such a database based on data curated from public domain sources.

Initial data collection efforts within NanoPUZZLES yielded a significant number of data points from various peer-reviewed publications. By extending the Klimisch criteria for toxicological data quality assessment, criteria for assessing the quality of data reported for nanomaterials, as well as the suitability of datasets for building QSARs, were developed. However, organising nanomaterial data remains a challenge. The current focus of data collection efforts within NanoPUZZLES is the exploration and evaluation of standards for organising experimental data for nanomaterials: the recently published ISA-Tab-Nano file format is of particular interest. The need for a unique identifier for nanomaterials and minimum information standards for a nanomaterials database is also being addressed.

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P5-02

Oxidant generating capacity as a metric to allow grouping of nanomaterials and prediction of human health effects (nanOxiMet)

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The surface of nanomaterials (NMs) and their ability to form oxidants, e.g. reactive oxygen species (ROS) are promising metrics to predict their toxicological potency as was shown in recent studies. An evaluation and testing of this hypothesis, with the aim to allow grouping of NMs based on their potential risk, will be pursued in the project nanOxiMet. Thus the focus of the investigation is on the physical properties of different NMs, their surface (re-)activity and toxicological responses in lung cells. Briefly summarized, the surface area and ROS generation potency of selected NMs will be analysed in liquids with four different methods in addition to standard NM characterizations e.g. size distribution and morphology. The toxicological studies will focus on the induction of oxidative stress and different stages of oxidative cellular responses on lung cells. Additional studies include a) the determination of oxidative damage to relevant macromolecules (e.g. proteins, genomic DNA), b) the investigation of oxidative cellular responses by activation of specific signalling pathways according to the level of oxidative stress for a full description of the target cells sensitivity to different NMs, and c) a systematic investigation of NMs with a broad range of reactivity using a large panel of biological endpoints ranging from initial intracellular ROS production to hierarchical oxidative stress responses and oxidative damages. The physical characteristics and toxicological results will be combined and evaluated in view of possible grouping based on the potency of the NMs induced adverse health effects. The grouping may then lead to a simplified development and market placement of new NMs.

SAFETY, CURRENT REGULATIONS AND SOCIAL/ETHICAL ASPECTS

P6-01

The DaNa2.0 knowledge base nanomaterials - Latest research results on the effects of nanomaterials on humans and the environment

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Nanotechnology is considered one of the key technologies of the 21st century. The success of this fascinating technology is particularly based on its versatility. It will bring about fundamental changes of basic research as well as of many sectors of industry and also of daily life from electronics to the health care system. However, many consumers miss reliable and understandable information on nanomaterials and nanotechnology, e.g. on the basic questions: What exactly are nanoparticles? What is meant by “exposure”? When do toxicologists speak of a risk?

These and many more questions are answered by our knowledge base www.nanoobjects.info.

In an interdisciplinary approach, scientists from different research areas such as human and environmental toxicology, biology, physics, chemistry and pharmacy provide a knowledge base for more transparency. The DaNa2.0 project team processes the results of latest research on nanomaterials regarding their influence on humans and the environment in an easily comprehensible way for interested laymen, stakeholders and scientists.

For this purpose, we analyse scientific publications, reports and latest news on human and environmental toxicology. The state of knowledge is wrapped up in the knowledge base. An integrated application-based database, the «slot-machine», allows for mapping between an application of nanoproducts, the material itself and possible outcome/toxicological effects. To facilitate the evaluation process of scientific publications, we developed a methodology, the so-called «Literature Criteria Checklist» as well as a Standard Operation Procedure (SOP) template based on careful scientific practice. Additionally, DaNa2.0 provides a list of FAQs as well as the opportunity to pose questions to our experts via mail. DaNa2.0 is also present on Twitter, follow us @nano_info.

DaNa2.0 is a German umbrella project funded by the German Federal Ministry of Education and Research and supported by Swiss Federal Authorities.

P6-02

Transnational access to VITO’s occupational exposure assessment facility

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The QNano Transnational Access (TA) element is dedicated providing users from the European nanosafety community access to nanomaterials processing, characterisation and exposure assessment facilities. Proposals for access may be submitted in response to calls. The calls and the application forms are available online (http://www.qualitynano.eu).

VITO Belgium offers their occupational exposure assessment facility for Transnational Access which includes:

- Instrumentation to determine particle concentration (number, surface area, charge, sampling) and particle size in air;
- Specific nano aerosol generators;
- An indoor aerosol test chamber.

Because field testing is difficult to arrange, expensive and usually uncontrolled, the instrumentation and test chamber can be used to:

- simulate processes (abrasion, wear and tear) and activities (nano powder handling) emitting nanoparticles;
- generate nano aerosols and study aerosol physics (e.g. dispersion, agglomeration);
- identify processes likely to produce highest occupational exposures
- characterize temporal and spatial variations of aerosol concentrations at specific nano processing
- evaluate performance of real-life engineering controls of nano aerosol
- (inter)compare instrumentation;
- optimize measurement methodologies and sampling techniques.

Specifications aerosol test chamber:

- Size: 4.9 (l) x 2.8 (w) x 2.6 (h) = 36 m3
- Negative pressure system (safety)
- Controllable flows (inlet/outlet/recirculation)
- HEPA filtered inlet (particle free) also adaptable to unfiltered inlet (background)
- HEPA filtered exhaust
- A panel perpendicular to the flow for injecting generated aerosol
- Panels for guiding through sample inlet tubes
- Possibility to split up into two rooms through movable panels with flow circulation
- Temperature and humidity monitoring and logging

The poster will give an overview of VITO\'s occupational exposure assessment facility.

P6-03

Nanotechnologies and regulation: impressions of the academy, the industry and the regulators in Brazil and Europe

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Good regulation for nanotechnology and nanomaterials is predicated on principles of general good regulatory governance. Nanotechnologies, like all technologies, certainly present risks, whether they result from unintentional effects of the beneficial applications, or from the malevolent misuse of the technology. Increasingly, risks from new and emerging technologies are being discussed at international level, although this initiative is only beginning to consider the appropriate responses to nanotechnology. This study attempts to examine the impressions of the academy, the industry and the regulator on regulating nanotechnologies in Brazil and Europe. Convergences have been identified for the different analyzed regulation questions, including the potential for cross-boundary harms, sharing of regulatory expertise and resources. However, there are also different points of views on the regulation form, which can be related to different perceptions of the risks and the risk management. The results suggest that increasing the international and intersectorial dialogues is essential to buildup regulatory frameworks able to meet health and innovation requirements.

OTHER TOPICS

OT-01

In vitro models of human biological barriers for safety testing of nanomaterials

Groß H.

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PharmBioTec GmbH is a Contract Research Organization offering high quality services in Biotechnological/Biopharmaceutical R&D during several stages in the drug development process (Figure 1). Our company operates in close cooperation with the Department of Pharmaceutical and Medicinal Chemistry and the Helmholtz Institute of Pharmaceutical Sciences (HIPS). It can revert to several decades of experience in design, synthesis, biological evaluation, formulation and analysis of new drugs.

Three professors (R. W. Hartmann, R. Müller and C. M. Lehr) have combined their extensive knowledge in different fields of pharmaceutical sciences to form a young and flexible company that facilitates free flowing innovation transfer between university, research institute and industry. PharmBioTec's main objective is to establish and apply new techniques in the areas of Biotechnology, Drug Discovery and Drug Delivery.

The department of Drug Delivery which is directly connected to the workgroup of Prof Claus-Michael Lehr (Helmholtz Institute of Pharmaceutical Research) offers a large range of cell culture- and tissue-based models of human biological barriers (in particular the gastro-intestinal tract, the skin and the lungs). These barrier models have been already successfully used in the scope of several European & international research projects focusing on the topic of nanosafety. In parallel PharmBioTec offers the formulation of multifunctional nanocarriers. These allow in vivo tracking of the system, targeting to the site of action, releasing the payload in a controllable manner and last but not least being safely biodegraded and excreted from the body. Prediction of drug resorption, biocompatibility and clearance leads to improvements in your drug formulation in terms of efficiency and tolerance.

OT-02

Nanotechnology in the Leibniz-Association - The Leibniz Network Nano

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The Leibniz Association is one of the four major science associations in Germany. It connects 86 independent research institutions that cover a wide range of scientific topics from the natural, engineering, life and environmental sciences via economics, spatial and social sciences to the humanities.

The Leibniz Network Nano is a network of meanwhile 15 institutes of the Leibniz Association. The network is coordinated by an office which is located at the INM - Leibniz Institute for New Materials in Saarbruecken.

Among other tasks the network is supposed to serve as a central point of contact in the area of nanotechnology. Furthermore it enables the collection and exchange of information between the partners and with external parties. It can also handle external requests for example from industry and provide contacts to suitable partners in the network. In addition it initiates and conducts joint projects of common interest of the partners and last but not least it performs joint activities such as workshops, conferences, exhibitions and more.

The partner institutes cover a large variety of competencies in nanotechnology. Already four major topics emerged so far: Surfaces with specific functional properties enable for example switchable adhesion, the immobilization of selected proteins or cells on surfaces or specific catalytic effects to name only a few. In the field of nanoelectronics, nanosensors and nanooptics new solutions for printed electronics, for chemical or magnetic sensors or for semiconductor modules with specific electronic properties are developed. Analytical methods and their potential for investigations on the nanoscale form another focus area, in which electron microscopy has a particular important role. Finally an important focus lies on nanomedicine, nanobiology and nanosafety.

This poster briefly describes the activities in nanotechnology within the Leibniz Association with special emphasis on the Leibniz Network Nano.

OT-03

Nanosafety: QM Based Analysis of Nanoproducts

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Twelve companies have joined forces in a regional network to develop QM based procedures for characterization of nanomaterials included in consumer and industrial products. The goal is to provide customers with standardized analytical and toxicological tests for a comprehensive assessment of the nanomaterial content in their products, e. g. to be accepted by regulatory and surveillance authorities. Furthermore, emphasis is put on method development to localize and characterize nanoparticles in water, food, cosmetics as well as in biological systems such as plant, animal or human bodies.

Core technologies are: (Surface) Mass spectrometry, Near field probes, Light microscopy, Flow and Scanning Cytometry, Electron microscopy, Holography, FFF, Molecular biology, Toxicology.

www.ncl-muenster.de

AUTHOR INDEX

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COVER PICTURES

Left side:
"Nanosafety" (INM – Uwe Bellhäuser, das Bilderwerk)
Top right:
Flowerlike In_2O_3 nanostructures (Karsten Moh, INM)
Bottom right:
A549 cell surface with adhering SiO_2 nanoparticles (Marcus Koch and Henrike Peuschel, INM)