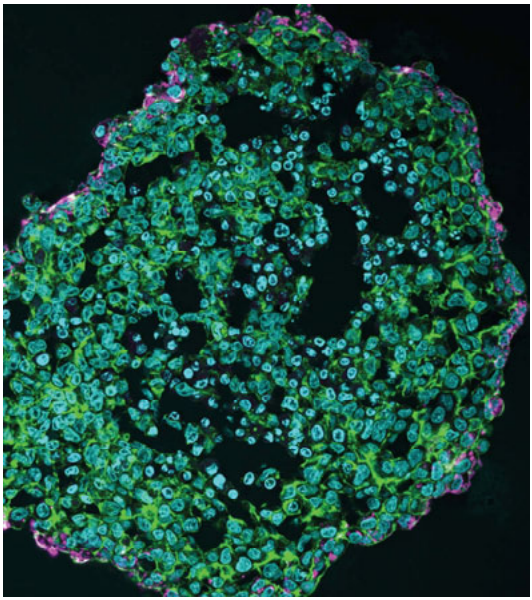

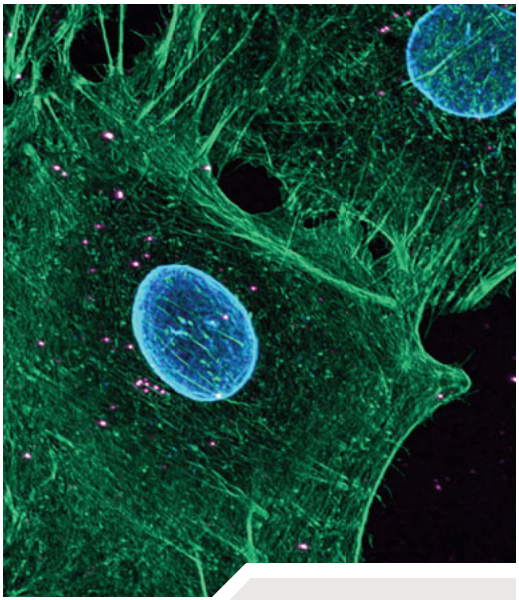

NANOSAFETY 2017

11–13 OCTOBER 2017
SAARBRÜCKEN, GERMANY

Leibniz Nanosafety

INM

NANOSAFETY 2017

organised by

INM – Leibniz Institute for New Materials

in association with
Leibniz Research Alliance Nanosafety

Saarbrücken, Germany, 11–13 October 2017

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PROGRAMME

WEDNESDAY, 11 OCTOBER 2017

08:00	Registration and coffee
09:30	Opening words
	SESSION 1 **Environmental Exposure Pathways** Chair: Christoph van Thriel
10:00	*Keynote:* "Evaluating the Environmental Health and Safety Implications of Engineered Nanomaterials" *William K. Boyes*, United States Environmental Protection Agency, Research Triangle Park, NC, USA
10:45	"Modeled Engineered Nanomaterial (CeO_2, SiO_2, Ag) Releases and Concentrations in Germany" *Fadri Gottschalk*, Institute of Safety and Risk Sciences (ISR), BOKU, Vienna, Austria
11:05	**Coffee break**
	SESSION 2 **Safe by Design** Chair: Annette Kraegeloh
11:25	*Keynote:* "High-Throughput for Toxicology and Material Discovery with Particle Technology" *Lutz Mädler*, Foundation Institute of Materials Science (IWT) and University of Bremen, Germany
12:10	"The Safe-By-Design Concept and a Corresponding Platform for Nanomaterials, Driven by the Requirements of Regulations and Other Needs" *Blanca Suárez Merino*, TEMAS AG, Zürich, Switzerland
12:30	"Lab-on-a-Chip-Based High-Throughput Screening of the Engineered Nanomaterial Toxicity" *Nicolas Voelcker*, Monash Institute of Pharmaceutical Sciences, Melbourne, Australia
12:50	**Lunch**

	SESSION 3 **Regulatory Issues and Long Term Effects** Chair: Heinz Fehrenbach
14:00	"Risk, Regulation and Responsible Innovation" *Maurice Brennan*, University of Birmingham, UK
14:20	"European Standardization in Nanotechnologies and Relation with International Work. How Standardization Can Help Industry and Regulators in Developing Safe Products?" *Patrice Conner*, AFNOR, La Plaine Saint-Denis, France
14:40	"NanoValid: Developing Reference Methods for Risk Assessment of Engineered Nanomaterials" *Rudolf Reuther*, NordMiljö AB, Arvika, Sweden
15:00	"Tuball ™ Single Wall Carbon Nanotubes: Health, Safety & Environmental Issues" *Gunther van Kerckhove*, OCSiAl Europe S.a.r.l., Leudelange, Luxembourg
15:20	**Coffee break**
15:40	POSTER SESSION 1

	SESSION 4.1 **Quantification and Detection of Nanoobjects** Chair: Annette Kraegeloh
16:45 – 17:20	*Invited:* "Labelled SiO_2 Nanoparticles as Markers of Contamination during Nanomaterial Handling Operations" *Jesús Santamaría*, Nanoscience Institute of Aragon, University of Zaragoza, Spain
19:15	**Conference Dinner** "Luminanz" Quartier Eurobahnhof, Europaallee 21, 66113 Saarbrücken

THURSDAY, 12 OCTOBER 2017

	SESSION 4.2 **Quantification and Detection of Nanoobjects** Chair: Eduard Arzt
09:00	*Keynote:* "Cryo Electron Tomography: Imaging Cells at the Nanoscale" *Wolfgang Baumeister*, Max Planck Institute of Biochemistry, Planegg, Germany
09:45	"Uptake Monitoring and Quantification of Nanomaterials at Single Cell Level by Means of Element- and Molecule Based Imaging and Dosimetric Techniques" *Irina Estrela-Lopis*, Institute of Medical Physics and Biophysics, University Leipzig, Germany
10:05	"Ultrasensitive Detection, Quantification and Identification of Engineered and Natural Nanoparticles Using Wide-Field Surface Plasmon Microscopy" *Vladimir Mirsky*, Institute of Biotechnology / Nanobiotechnology, Brandenburg University of Technology, Senftenberg, Germany
10:25	"Synthesis and Functionalisation of Bright Fluorescent Silica Nanoparticle Probes for High-Resolution STED and Confocal Microscopy" *Isabella Tavernaro*, INM – Leibniz Institute for New Materials, Saarbrücken, Germany
10:45	**Coffee break**
	SESSION 4.3 **Quantification and Detection of Nanoobjects** Chair: Nicolas Voelcker
11:05	*Invited:* "Radiolabeling – An Appropriate Tool to Study the Environmental Fate of Engineered Nanoparticles" *Karsten Franke*, Helmholtz-Zentrum Dresden-Rossendorf, Germany
11:40	"Single Cell Level Quantification of Nanoparticle-Cell Interactions Using Mass Cytometry" *Angela Ivask*, Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia
12:00	"Characterization of Unlabeled Nanomaterials in Complex Environments" *Melinda Bartok*, Schaefer Technologie GmbH, Langen, Germany
12:20	**Lunch**

	SESSION 5.1 **Neurotoxicity** Chair: Klaus Unfried
13:20	*Invited:* "Air Pollution, Microglia, and the Lung-Brain Axis" *Michelle L. Block*, The Stark Neuroscience Research Institute, Indianapolis, Indiana, USA
13:55	"Investigation of Neurotoxic Effects of Manganese Nanoparticles in 3D Neural Microtissue" *Christoph van Thriel*, Leibniz Research Centre for Working Environment and Human Factors (IfADo), Dortmund, Germany
14:15	"Development of an *in vitro* Model of NanoNeurotoxicity Using Human 3D Neural Tissues" *Luc Stoppini*, Hepia/HES-SO, Univ. of Appl. Sciences Western Switzerland, Geneva
14:35	**Coffee break**
	SESSION 5.2 **Neurotoxicity** Chair: Christoph van Thriel
14:55	"A Daily Exposure of Rabbit Dams to Diesel Exhaust Nanoparticles during Gestation Impairs the Brain and Olfactory Dopaminergic Pathways of Offspring" *Estefania Bernal-Meléndez*, URAFPA, INRA UC340, University of Lorraine, Vandoeuvre-les-Nancy, France
15:15	"Effects of Nanoparticles in the 5xFAD Mouse Model of Alzheimer's Disease" *Catrin Albrecht*, IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
15:35	POSTER SESSION 2
16:40 – 18:00	WORKSHOPS 1 - Safe by Design 2 - Neurotoxicity 3 - Communicaton of Safety and Risks

FRIDAY, 13 OCTOBER 2017

	SESSION 6.1 **Nanomaterials: Effects and Mechanisms** Chair: Heinz Fehrenbach
09:00	*Invited:* "The Effect of Gold Nanoparticles in Asthma Mouse Model" *Thai Dinh*, Universitätsklinikum des Saarlandes, Saarbrücken, Germany
09:35	"The Impact of CuO and TiO_2 Nanoparticles on Gene Expression Following Air-Liquid Interface Exposure" *Matthias Hufnagel*, Karlsruhe Institute of Technology, Germany
09:55	"How the Protein Corona Evolves on a Nanoparticle" *Giancarlo Franzese*, Física de la Matèria Condensada, Universitat de Barcelona, Spain
10:15	"Low-Dose Carbon Black Nanoparticles Exposure of the Lung Does Not Aggravate Allergic Airway Inflammation in Mice" *Heinz Fehrenbach*, Priority Area Asthma & Allergy, Research Centre Borstel, Germany
10:35	**Coffee break**
	SESSION 6.2 **Nanomaterials: Effects and Mechanisms** Chair: Klaus Unfried
10:55	"Understanding Nanosafety – The Importance of Assay Performance *in vitro"* *Cordula Hirsch*, Empa, St. Gallen, Switzerland
11:15	"Exposure to Nanoparticles Specifically Extends Neutrophilic Life Span: A Toxicologically Relevant Endpoint for Nanoparticle Safety" *Tamara Hornstein*, IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany
11:35	CONCLUSION
	Workshop presentations and final discussion Poster award Closing remarks
12:30	**Brown bag lunch**
14:00-16:00	INM GUIDED TOUR (optional, registration on-site)

▶ SESSION 1

Environmental Exposure Pathways

O1-1 invited

EVALUATING THE ENVIRONMENTAL HEALTH AND SAFETY IMPLICATIONS OF ENGINEERED NANOMATERIALS

William K. Boyes

US Environmental Protection Agency, Research Triangle Park, NC USA

Engineered nanomaterials (ENM) are a fundamental and growing component of the global economy, and are projected to reach an annual economic impact in the hundreds of billions of dollars. Currently, their growing use far outpaces our ability to evaluate potential for adverse impacts on environmental health and safety. We recently proposed a framework to evaluate the health and safety implications of ENM releases into the environment, including purposeful releases such as for antimicrobial sprays or nano-enabled pesticides, and inadvertent releases as a consequence of other intended applications. Considerations encompassed potential releases of ENM to the environment across product life cycles, fate, transport and transformations in environmental media, exposed populations, and possible adverse outcomes. This framework was structured as a series of compartmental flow diagrams to guide future development of quantitative predictive models, identify research needs, and support development of tools for making risk-based decisions. If released, ENM are not expected to remain in their original form due to reactivity and/or propensity for hetero-agglomeration in environmental media. Therefore, emphasis was placed transformations of ENM that might occur in environmental or biological matrices. Predicting the activity of ENM is difficult due to the multiple dynamic interactions between the physical/chemical aspects of ENM and similarly complex environmental conditions. Therefore, the use of simple predictive functional assays was proposed as an intermediate step to address the challenge of predicting environmental fate and behavior of ENM. The nodes of the proposed framework reflect phase transitions that could be targets for development of such assays. Application, refinement, and demonstration of the framework, along with an associated knowledgebase that includes targeted functional assay data, will someday allow better de novo predictions of potential ENM exposures and adverse outcomes. Only by developing an efficient ability to forecast and avoid potential environmental health and safety problems across the life cycle of ENM development, use and disposal, can we fully realize the many potential societal benefits promised by the nanotechnology revolution.

This is an abstract of a proposed presentation and does not reflect EPA policy.

O1-2

MODELED ENGINEERED NANOMATERIAL (CEO_2, SIO_2, AG) RELEASES AND CONCENTRATIONS IN GERMANY

Fadri Gottschalk[1], Bernd Giese[2] and Michael Steinfeldt[3]

[1] Engineering, Technical and Scientific Services, ETSS AG, Strada, Switzerland
[2] University of Natural Resources and Life Sciences (BOKU), Institute of Safety and Risk Sciences (ISR), Vienna, Austria
[3] Universität Bremen, Faculty of Production Engineering, Department of Technology Development and Design, Bremen, Germany

A growing number of products and processes involving engineered nanomaterials (ENM) has been developed during the past decades and due to the unique qualities of present and potential new ENM a strong growth of production is assumed in medium–term forecasts [1]. Despite efforts to limit the losses during manufacture, transport, use and disposal, the release volumes of ENM will increase accordingly. An assessment of current and potential future exposure is therefore indispensable as an accompanying monitoring process. The case of food-grade titanium dioxide shows that widespread use of an ENM does not guarantee that it poses no health risk [2].

In this contribution we will present results of a model for present and future exposure to CeO_2-, SiO_2- and Ag-ENM in Germany. CeO_2-, SiO_2- and Ag-ENM on the one hand are chosen because of its distributed use in a number of different applications and on the other hand due to some potentially important diffuse releases of ENM connected with some of these applications. In order to assess exposure and corresponding risk, the present work includes an estimation of the current and future use and release volumes, a probabilistic model for the calculation of current and future environmental concentrations (PEC) in technical as well as natural compartments and an estimation of the potential risks emanating from CeO_2-, SiO_2- and Ag-ENM in waterbodies.

Our fully dynamic simulation of ENM life cycles takes into account differences in the input (production volumes) as well as a varying distribution inside the system due to product storage, multi-year life cycles and differences in transport and stability. For this purpose three stochastic models are designed for a) ENM release, b) ENM circulation (delayed release) and c) ENM volumes in nature and technical sinks over a large time period of several decades.
Thereby accuracy of present and future mass flows and environmental concentrations is significantly improved. To enable an estimation of potential risks due to hazardous effects in waters, a preliminary risk assessment was conducted by analyzing the overlap of PEC and a probabilistic species sensitivity distribution for the respective ENM [3][4].

[1] Ricardo Energy & Environment, Workshop Working Material, Report for European Commission DG Environment (2016) p. 10.
[2] S. Bettini et al., Scientific Reports 7, 40373 (2017), p. 1-13.
[3] F. Gottschalk and B. Nowack, Integrated Environmental Assessment and Management 9 (2012), p. 79-86.
[4] The authors acknowledge funding from the German Federal Ministry of Education and Research (BMBF) in the context of the project DENANA - Design criteria for sustainable nanomaterials (grant number: 03X0152).

SESSION 2

Safe by Design

O2-1 invited

HIGH-THROUGHPUT FOR TOXICOLOGY AND MATERIAL DISCOVERY WITH PARTICLE TECHNOLOGY

Lutz Mädler

Foundation Institute of Materials Science (IWT), Department of Production Engineering,
University of Bremen, Germany

High-throughput screening is a well-established method for scientific experimentation in chemistry and biology. Examples are heterogeneous catalysts, drug developments and nanoparticle toxicology. These methods involve the synthesis of small sample volumes often in form of particles that are quickly tested. These tests are designed to quickly obtain easily accessible data (called descriptors) that are related with a predictor function to the desired properties. The descriptor-predictor-relation is found through mathematical modelling and calibration. One particle based high-throughput concept for the evaluation of potential toxicological hazards will be presented in more detail. Furthermore, a new concept is presented which transfers high-throughput screening to the exploration of new structural metals.

O2-2

THE SAFE-BY-DESIGN CONCEPT AND A CORRESPONDING PLATFORM FOR NANOMATERIALS, DRIVEN BY THE REQUIREMENTS OF REGULATIONS AND OTHER NEEDS

Andreas Halbleib[1], Til Schlotter[2] and Karl Höhener[3]

[1] Owner and CEO of TEMAS AG, Zürich Switzerland
[2] Project leader Safe-by-design of TEMAS AG, Zürich Switzerland
[3] Advisor of the CEO of TEMAS AG, Zürich Switzerland

Being one of the most promising technologies in the last decade, researchers and industry are taking advantage of nano-sized materials, bringing new materials and products to the market. A concept that is already used in other industry sectors, is the Safe-by-Design (SbD) concept. The TEMAS AG and RIVEM adapted and further developed the concept for nanomaterials within the EU projects NANoREG and Prosafe.
The SbD platform helps innovators to be prepared for regulations; thus, it established transparency and therefore trust between innovators, and consumers. The final goal of the application of the SbD concept is to design safer nanomaterials and nanoproducts, apply the precautionary principle, identify uncertainties and risk potentials at the earliest possible point in time, and actively manage them. This can be achieved since the SbD concept is based on regulatory requirements, but enabling the user to work independently and self-determined at each phase of an innovation and development project.
In order to help an innovator to navigate through and correctly apply the SbD concept, a Web based SbD Implementation Platform has been developed by TEMAS. The platform is already being tested together with selected partners from industry. It is designed in such a way that the innovation phases and their needs can be individually adjusted to each project. Like this innovators, regulators, and consumers can live together in a safer and much more transparent "environment".

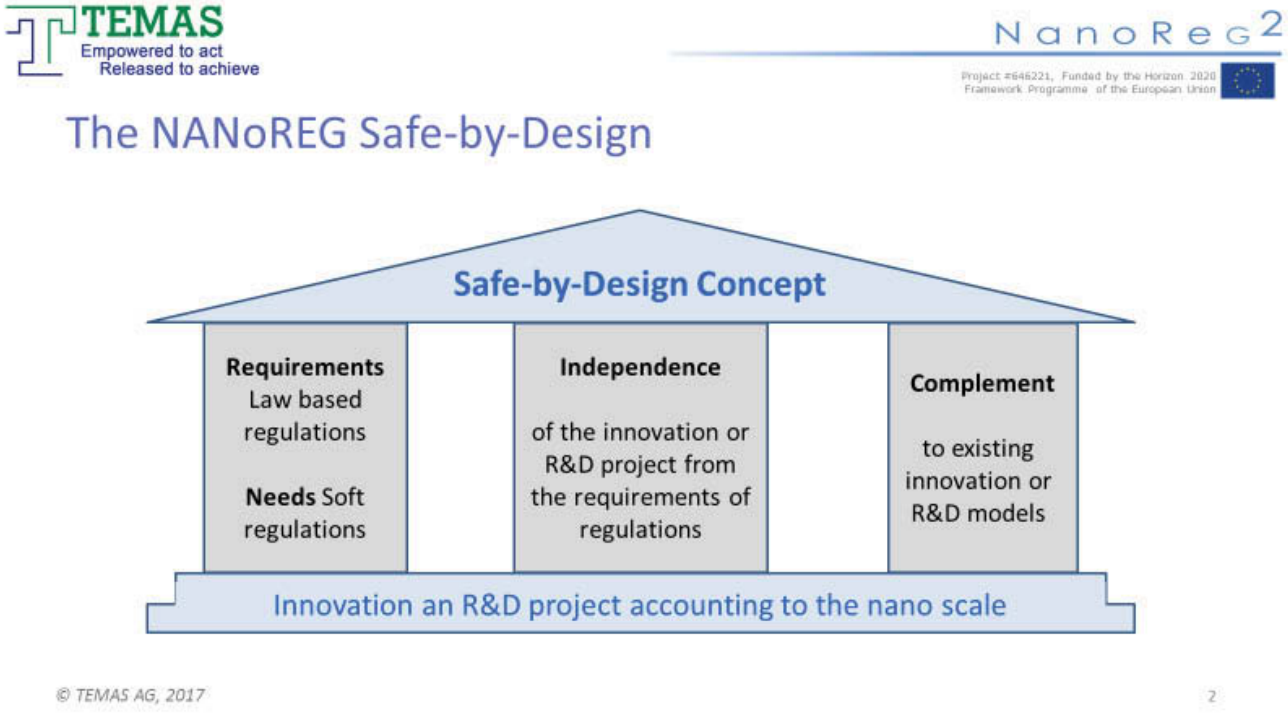

Figure 1. The NANoREG Safe-by-Design foundation and pillars .

O2-3

LAB-ON-A-CHIP-BASED HIGH-THROUGHPUT SCREENING OF THE ENGINEERED NANOMATERIAL TOXICITY

Ziqiu Tong[1,2], Angela Ivask[2], Scott McCormick[2], Enzo Lombi[2], Craig Priest[2], Nicolas H. Voelcker[1-5]

[1] Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC, Australia
[2] Future Industries Institute, University of South Australia, Mawson Lakes, South Australia, Australia
[3] Leibniz Institute for New Materials, Saarbruecken, Germany
[4] Melbourne Centre for Nanofabrication, Victorian Node of the Australian National Fabrication Facility, Clayton, VIC, Australia
[5] Monash Institute of Medical Engineering, Monash University, Clayton, VIC, Australia

Here, we report on lab-on-a-chip platforms developed in our lab for the multiparametric analysis of the effects induced by engineered nanomaterials (ENMs)
The rapid growth of nanotechnology and the concomitantly increased rate of synthesis of ENMs has meant that ENMs are becoming ubiquitous in our environment [1]. As a consequence, the risk for living organisms coming into contact with ENMs that are still not been well-defined in term of toxicological profile is increasing. In addition, recent studies that have revealed genotoxic risks associated with ENMs have further exacerbated this situation, highlighting the urgent necessity to better define the possible toxicological effects of ENMs and their physico-chemical characteristics. However, the combinatorial diversity of nanomaterials makes their rapid toxicological classification difficult without the application of high-throughput screening (HTS) approaches.
The first HTS platform is based on the cytokinesis-block micronucleus (CBMN) assay [2,3], lab-on-chip cell sorting and automated image analysis, has been successfully applied in the evaluation of the cytotoxic and genotoxic effects induced by AgNPs and SiO_2NPs, and the role of their physico-chemical properties such as composition, surface coating, size and surface charge. In particular, our results demonstrate the high cyto- and genotoxicity induced by AgNPs and the biocompatibility of SiO_2NPs, both administered at the same dose in primary human lymphocytes, emphasising the importance of ENMs composition in the toxicity outcome. Moreover, our data highlight that the toxic effects are dependent on dose, size and coating.
The second HTS platform involves the use of crossed laminar flow microfluidics for the selective capture of multiple cell types on chip aiming for high throughput screening of various cell treatment compounds [4]. Parallel laminar streams containing different cell types can be perfused and captured on cell adhesion protein-functionalized reaction area. Thereafter, parallel streams containing nanoparticle tsolutions are delivered orthogonally over the captured cells. Multiple cell types and a range of cell treatment conditions can therefore be assessed in a single experiment. We also demonstrate sorting of mixed cell populations via antibody array clusters, and to further deliver treatments to subpopulations of cells. This crossed laminar flow microfluidics offers an exciting platform for high throughput screening of nanoparticle toxicity testing.

[1] Malysheva, A.; Lombi, E.; Voelcker, N.H. et al. Bridging the divide between human and environmental nanotoxicity. *Nature Nanotech.* 2014, 10, 835-844.
[2] Anglin, E. J.; Salisbury, C.; Bailey, S.; Hor, M.; Macardle, P.; Fenech, M.; Thissen, H.; Voelcker, N. H., Sorted cell microarrays as platforms for high-content informational bioassays. *Lab Chip* 2010, *10* (24), 3413-3421.
[3] Fenech, M., Cytokinesis-block micronucleus cytome assay. *Nat. Protoc.* 2007, *2* (5), 1084-104.
[4] Tong Z., Ivask A., Guo K., McCormick S., Lombi E., Priest C., Voelcker, N.H. Crossed flow microfluidics for high throughput screening of bioactive chemical-cell interactions. *Lab Chip.* 17 (2017), 501 – 510.

SESSION 3

Regulatory Issues and Long Term Effects

O3-1

RISK, REGULATION AND RESPONSIBLE INNOVATION

Maurice Brennan

School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, United Kingdom

The European Union (EU) adoption of strategic, scientific and regulatory frameworks for nanomaterials have an important role to play in protecting human and environmental health and safety. There is an ambition within the EU to realise the full benefits of the nano revolution, albeit with more desirable futures, with a policy momentum to set Responsible Research and Innovation (RRI) at the heart of the innovation process. This presentation challenges to what extent the EU risk governance policy and instruments have impacted on achieving its strategic goal for the safe and responsible development of nanomaterials. Results are presented from a qualitative research study performed to evaluate cross-sectoral expert assessments on the progression towards achieving the above strategic goal and the key objectives set out in the European Commission(EC) Nanomaterials Risk Governance Strategy. The main findings identify that nanomaterials currently overflow their regulatory boundaries due to the lack of nano specificity in the EU horizontal chemical safety regulations and risk assessment testing protocols. That whilst there is cross-sectoral recognition of RRI, its interpretive flexibility has meant the implementation of the concept of responsible innovation is inconsistent with little indication of effective industry engagement, especially by small and medium size companies. Consequently, the EC strategic goal and objectives are assessed as not having yet been met. But there are sign postings that emergent scientific risk assessment protocols can provide nano-specificity to underpin regulatory testing regimes, and the EC promotion of the Safety by Design process concept could operationalize RRI within the innovation value chain for nanomaterials.

O3-2

EUROPEAN STANDARDIZATION IN NANOTECHNOLOGIES AND RELATION WITH INTERNATIONAL WORK. HOW STANDARDIZATION CAN HELP INDUSTRY AND REGULATORS IN DEVELOPING SAFE PRODUCTS?

Patrice Conner

AFNOR Standardization, Management and Consumer Services Department, La Plaine Saint-Denis, FRANCE. Also Secretary of CEN/TC 352 "Nanotechnologies"

Nanotechnologies have enormous potential to contribute to human flourishing in responsible and sustainable ways. They are rapidly developing field of science, technology and innovation. As enabling technologies, their full scope of applications is potentially very wide. Major implications are expected in many areas, e.g. healthcare, information and communication technologies, energy production and storage, materials science/chemical engineering, manufacturing, environmental protection, consumer products, etc. However, nanotechnologies are unlikely to realize their full potential unless their associated societal and ethical issues are adequately attended. Namely nanotechnologies and nanoparticles may expose humans and the environment to new health risks, possibly involving quite different mechanisms of interference with the physiology of human and environmental species.

One of the building blocks of the "Safe, integrated and responsible" or "Safe by design" approach is standardization. Both the Economic and Social Committee and the European Parliament have highlighted the importance to be attached to standardization as a means to accompany the introduction on the market of nanotechnologies and nanomaterials, and a means to facilitate the implementation of regulation. ISO and CEN have respectively started in 2005 and 2006 to deal with selected topics related to this emerging and enabling technology.

In the beginning of 2010, EC DG "Enterprise and Industry" (now EC DG GROWTH, Internal Market, Industry, Entrepreneurship and SMEs) addressed the mandate M/461 to CEN, CENELEC and ETSI for standardization activities regarding nanotechnologies and nanomaterials. Thus CEN/TC 352 "Nanotechnologies" has been asked to take the leadership for the coordination in the execution of M/461 (46 topics to be standardized) and to contact relevant European and International Technical committees and interested stakeholders as appropriate (56 structures have been identified). Prior requests from M/461 deal with characterization and exposure of nanomaterials and any matters related to Health, Safety and Environment.

Answers will be given to:

- What are the structures and how they work?
- Where are we right now and how work is going from now onwards?
- Are there standards on quantification, detection and identification of nano-objects?
- What are the relations with other Technical Committees especially with CEN/TC 137 "Assessment of workplace exposure to chemical and biological agents" and CEN/TC 195 "Air filters for general air cleaning"?
- What are the links between Research and Standardization projects?
- How CEN's work and targets deal with and interact with global matters in this field?

O3-3

NANOVALID: DEVELOPING REFERENCE METHODS FOR RISK ASSESSMENT OF ENGINEERED NANOMATERIALS

Rudolf Reuther

NordMiljö AB (NOMI), Arvika, Sweden

The growing production and commercialization of engineered nanomaterials (EN) will increase exposure of man and the environment to these novel substances. Their great innovation potential consists in fundamentally new properties that occur at nano-scales (1-100 nm), which may also trigger the changes we observe in their biological and environmental behavior. Despite a decade of world-wide intense research, we still lack a clear and consistent perception of what is going on when EN enter living systems, partly because current measurement and testing tools developed for conventional chemicals often do not reflect particle size dependent properties and possible associated effects. The resulting data uncertainty has provoked the urgent need to develop validated methods that can help to improve existing material and knowledge databases and our current understanding on critical material characteristics and mechanisms that control the interaction of nanoparticles with living systems, and to make regulatory risk approaches applicable to EN.

NanoValid was the first European "flagship" project entirely devoted to the development of validated reference methods and materials needed to reliably characterize size related properties, hazards and exposure of EN. Beside the development of a wide range of well characterized reference tools, such as rigorously validated standard operation procedures (SOPs) or certified reference materials (CRM) that can support risk assessors in industry and regulatory bodies to recognize and manage possible risks arising from nanomaterials, NanoValid created an enormous wealth of new scientific knowledge on the behavior of these new substances in man and natural systems. New insight was produced on critical dose-response relationships, toxic (adverse) pathways and end-points as well as on processes that govern the release, distribution, bioavailability and uptake of nanoparticles in biological systems along various life cycle stages.

Based on these new scientific findings, NanoValid generated also new practical and commercially exploitable sampling, measurement and testing instruments. In particular a novel online exposure device that combines physical and biological assays for airborne particles and a new hot gas nano-sampler has been developed. In cooperation with standardization bodies, such as CEN and ISO, NanoValid provided a strong input to current standardization efforts, such as CEN/TC 352 "Nanotechnologies", ISO/TC 229 "Nanotechnologies", or ISO/TC 24/SC 4 "Particle characterization", by proposing new work items (NWI) for method standardization. Another highlight and direct practical outcome of the project was the preparation of a guidance document and training manual for the safe handling of nanomaterials that can be used by small industries, such as research companies, instrument and material manufacturers or enforcement labs, to help to prevent, reduce and minimize any occupational health and safety risks.

The newly developed methodology, in particular the validated SOPs on size distribution or surface charge measurement, dispersion control, labeling and *in vitro / in vivo* testing, will strengthen future method validation efforts going on in other relevant projects and hence contribute to the generation of a reliable database that we urgently need to improve the performance of current risk and life cycle assessment tools and hence the enforcement of relevant legislations, such as REACH. The use of these validated methods will also help to early identify critical processes, material properties and life cycle stages, and promote the design of safe nanomaterials, to finally prevent any harm to man or the environment ("safety by design").

O3-4

TUBALL ™ SINGLE WALL CARBON NANOTUBES: HEALTH, SAFETY & ENVIRONMENTAL ISSUES

Gunther Van Kerckhove

OCSiAl Europe S.a.r.l., Leudelange, Grand Duchy of Luxembourg

The company OCSiAl has been founded in 2009 with a worldwide footprint. The first manufacturer of SWCNT with a patented scalable production process since 2013 and is for the moment the world's biggest SWCNT producer with 10t/year since 2015. Different product lines containing Tuball™ that are available @ OCSiAl or its partners. At extremely low loadings of Tuball™ SWCNT's, starting at just 0.02% (mass %), can provide the required electrical conductivity and simultaneously retain the mechanical properties of this Tuball™ product.

OCSiAl is also the first SWCNT manufacturer who has completed his EU-REACH registration for a tonnage band of up to 10T/a. Because Tuball™ is used and also tested in various applications on an ongoing basis, also receiving a lot of interests worldwide. That is why it is obvious that the company OCSiAl is establishing the necessary regulatory and quality standards worldwide.

The first part of this presentation will aim at providing a short introduction of our Tuball™ substance and his product line, a second part of the presentation will be an overview of the status and plans of the ongoing registrations an compliance. The third and last part of the presentation will focus on the health, safety and environmental aspects of our Tuball™ substance and the different applications.

As SWCNT manufacturer, OCSiAl is doing continues investments in improving our understanding of our different (new) Tuball products themselves and potential hazards through their (entire) life cycle. We are involved in generating additional test data and collaborating with industry associations and networks.

This presentation will describe the steps being taken by the company H&S Lead manager, Van Kerckhove Gunther to successfully introduce our carbon nanotubes (SWCNT's) regulatory status and outline our (future) plans for numerous of studies and qualifying our Tuball™ substance including the different kind of compositions.

▶ SESSION 4

Quantification and Detection of Nanoobjects

O4-1 invited

LABELLED SIO_2 NANOPARTICLES AS MARKERS OF CONTAMINATION DURING NANOMATERIAL HANDLING OPERATIONS

Alberto Clemente[1], M. Pilar Lobera[1], Francisco Balas[1,2] and Jesus Santamaria[1,2]

[1] Dept. Chemical Engineering, Aragon Institute of Nanoscience (INA), University of Zaragoza, Zaragoza, Spain
[2] Networking Biomedical Research Center (CIBER), Madrid, Spain

Almost every operation involving the handling of dry nanoparticles has the potential of generating a nanoparticle aerosol. Common industrial (e.g. opening containers, cleaning filters, emptying synthesis vessels), and laboratory operations (e.g. pouring, mixing, grinding, weighting), are likely sources for emission of engineered nanoparticles. Of concern to health and safety workers is not only the possibility of inhalation of nanoparticles, which can be minimized by adopting the required safeguards, but also the contamination of work surfaces, utensils and clothing, which is often unavoidable. Determining this contamination is further complicated by the fact that engineered nanoparticles are only a small proportion of background nanosized matter and therefore direct observation by highly sensitive techniques such as electron microscopy can generally be ruled out as a method to quantify exposure.
An easy-to-implement method to detect this contamination is sorely needed. Ideally, the method used should not interfere with the operations being carried out, should be cost-effective and robust. Here we present a new detection procedure based on fluorescent silica nanoparticles that are highly luminescent, thereby providing a sensitive signal when confronted with light of the appropriate wavelength. Some examples are shown below. To detect contamination in a given operation, a small amount of these nanoparticles would be added to the material being handled, and the potential contamination could then be easily assessed by simple illumination with a portable, low-power laser source.

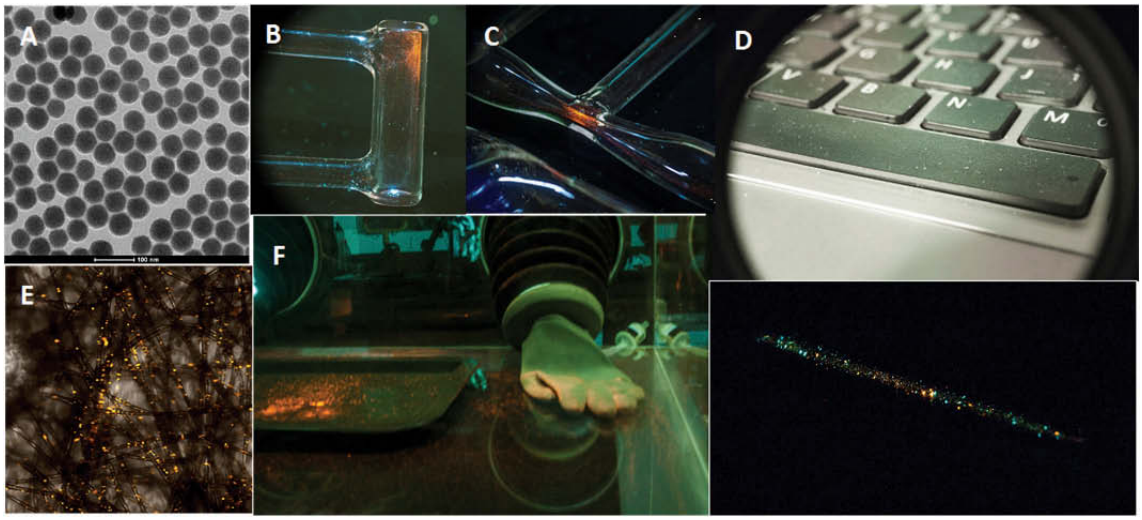

Figure 1. A) TEM image of the nanoparticles used as tracers. B-F: Examples of detection of contamination by nanoparticles. B,C) contamination in glassware after flowing a nanoparticle-containing aerosol. D) Spacebar of a keyboard under natural light (top) and under laser illumination. E) Fibers inside an HEPA filter after filtering a nanoparticle aerosol. F) Contamination in gloves and work surfaces inside a glove box after transferring 200 mg of nanoparticles between two vessels.

O4-2 invited

CRYO ELECTRON TOMOGRAPHY: IMAGING CELLS AT THE NANOSCALE

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Traditionally, structural biologists have approached cellular complexity in a reductionist manner by characterizing isolated and purified molecular components. This 'divide and conquer' approach has been highly successful, as evidenced by the impressive number of entries in the PDB. However, awareness has grown in recent years that only rarely can biological functions be attributed to individual macromolecules. Most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed *in situ*, i.e. in unperturbed cellular environments. Sensu stricto the term 'structural biology *in situ'* should apply only to a scenario in which the cellular environment is preserved in its entirety. Cryo electron tomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the power of three-dimensional imaging with the best structural preservation that is physically possible. Key methods developments, such as correlative LM/EM, Focussed Ion Beam Milling (cryo FIB) or phase plate imaging, will be discussed as well as applications highlighting the potential of this post-reductionist approach to structural biology.

O4-3

UPTAKE MONITORING AND QUANTIFICATION OF NANOMATERIALS AT SINGLE CELL LEVEL BY MEANS OF ELEMENT- AND MOLECULE BASED IMAGING AND DOSIMETRIC TECHNIQUES

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The degree and the mechanism of uptake, localization and distribution of nanomaterials (NMs) in cells and organs are major issues concerning toxicity and risk assessment of these novel products. Furthermore, the application of NMs as devices for diagnostic and therapeutic purposes requires monitoring of their interaction with cells. Label-free imaging techniques are therefore required.
The translocation of NMs across plasma membranes was studied in cancer culture cells *in vitro* as well in lung tissues of exposed rats *in vivo* by means of label-free imaging methods based on element and molecule analysis (Fig.1). The two ion beam techniques as proton induced X-ray emission (μPIXE) and Rutherford backscattering (μRBS) were used simultaneously at the Leipzig Ion Nanoprobe LIPSION for cell analysis. These two IBM techniques provide unique and powerful tools for element dosimetry and spatially resolved elemental analysis. The results of NM uptake *in vitro* were compared with *in vivo* study providing a tool for estimating the relevance of in vitro data for in vivo predictions. A quantitative analysis of minor and trace elements and their alterations due to NP loading at single cell level were performed and correlated with the cellular adverse response.
Furthermore NM subcellular localization and co-localization with cell compartments and biomolecules were investigated using confocal Raman microspectroscopy (CRM). CRM live imaging was additionally performed to follow the intracellular fate of nanoparticles. The uptake kinetics as well as the intracellular dissolution behavior were studied as well. Specific cellular regions of interest (nucleus, cytoplasm, lipid bodies/droplets, endoplasmic reticulum, mitochondria etc.) have been screened for spectroscopic signatures by means of cluster analysis or principle component analysis. The early alterations of chemical fingerprints in cells upon exposure to nanomaterials were evaluated as chemical biomarkers regarding their predictive potential.

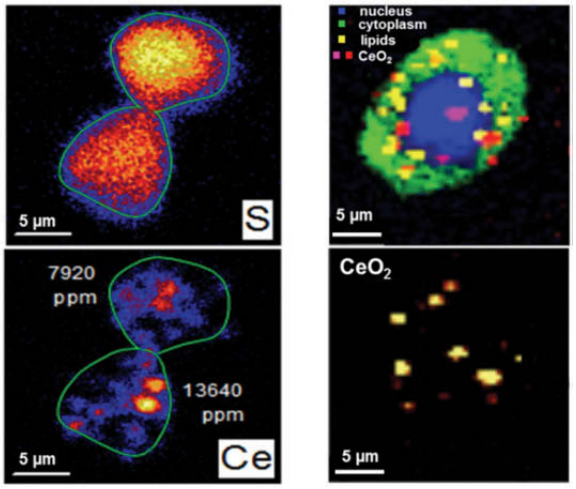

Figure 1. Label-free imaging of the distribution of CeO_2 nanoparticles in lung cancer cells by means of element (μPIXE: left) and molecule based (CRM: right) imaging techniques.

O4-4

ULTRASENSITIVE DETECTION, QUANTIFICATION AND IDENTIFICATION OF ENGINEERED AND NATURAL NANOPARTICLES USING WIDE-FIELD SURFACE PLASMON MICROSCOPY

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Permanent grow in the production of engineered nanoparticles increases a risk of human contacts with these potentially hazardous materials. It makes important a development of sensitive analytical methods for quantification and identification of nanoparticles in liquid and gaseous media. For this purpose, a method based on the detection and analysis of minute signals of surface plasmon resonance (SPR) images due to adsorption of single nanoparticles was developed [1-4]. This new technology allows one a real-time detection of interaction of single nanoparticles with sensor surface. Adsorption of each nanoparticle leads to characteristic diffraction image whose shape and intensity depends on the size and chemical composition of nanoparticle. A number of the nanoparticle–surface binding events per time and surface area characterizes volume concentration of nanoparticles. A large monitored surface area of sensor surface allows one to detect many hundreds events in each frame, this leads to a very high dynamic range of counting and to a correspondingly high dynamic range in the concentration scale. Depending on the type of nanoparticles and experimental conditions, the detection limit for aqueous samples can be below 1000 nanoparticles per microliter. Statistical analysis of images of nanoparticles provides information on heterogeneity of nanoparticles and can be used as fingerprints for identification of different types of nanomaterials. Chemical functionalization of the sensor surface as well as changes of pH or ionic strength are additional factors influencing the behavior of nanoparticles and allowing one their identification [3]. Independent information on chemical composition and size of nanoparticles can be obtained from SPR visualization of their electrochemical dissolving or modification during potential sweep [2]. The method was also applied for ultrasensitive detection and analysis of nanoparticles in very complex media, such as tap water, sunscreen, juice or wine [4]. The technology can be applied for engineered or for natural (i.e., exosomes) nanoparticles. Beside analytical applications, the new approach provides valuable scientific information on adsorptive properties of nanoparticles which can be used to predict their toxicity or to study interaction of nanoparticles with surfaces.

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[4]S. Nizamov, V. Scherbahn, V. M. Mirsky. Detection and quantification of single engineered nanoparticles in complex samples using template matching in wide-field surface plasmon microscopy, Anal. Chemistry 2016, doi: 10.1021/acs.analchem.6b02878.

O4-5

SYNTHESIS AND FUNCTIONALISATION OF BRIGHT FLUORESCENT SILICA NANOPARTICLE PROBES FOR HIGH-RESOLUTION STED AND CONFOCAL MICROSCOPY

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In recent years, the application of engineered nanomaterials in the biomedical field has gained more and more attention. Due to their remarkably high surface-to-volume ratio in combination with their unique properties, engineered nanomaterials are highly interesting candidates for applications in cancer treatment, drug delivery or labelling and sensing [1]. The localisation of nanoparticles by advanced imaging techniques combining resolution at the nanoscale (< 100 nm) with the potential for analysis of dynamic processes in living cells is of great relevance. In this context, techniques for the preparation of well-defined nanomaterials that can be labelled by versatile approaches are indispensable.

We present an effective method for the preparation of fluorescent silica nanoparticles in a size range between 15 nm and 80 nm that are applicable for STED (Stimulated Emission Depletion) and confocal microscopy. The tailored synthesis allows the embedding of different kinds of fluorophores (e.g., fluorescent dyes or proteins) and provides the option to gain multicolour silica nanoparticles. A multivalent presentation of bioactive ligands can be achieved by postsynthetic surface modification.

The obtained nanoparticles were compared to particles from commonly known synthesis methods, indicating a better control over the size distribution and particle morphology [2]. Analysis of TEM images and DLS measurements showed no influence of the fluorophore concentration on the particle size or the degree of agglomeration, whereas leaching experiments revealed a stable embedding of the fluorophores into the silica matrix. The spectroscopic characteristics of the fluorescent nanoparticles were similar to the behaviour of the free uncoupled fluorophores, but indicated higher quantum yields and better photostability. All nanoparticle suspensions were stable in water and biological medium.

These results are relevant to follow up the interaction mechanisms of nanoparticles using colloidal labelled silica as model for engineered nanomaterials in systematic assessment of the influence of size and other physico-chemical parameters on uptake and localisation studies. For example, the fluorescent nanoparticles have been used to determine their intracellular agglomeration and nuclear penetration after uptake into Caco-2-cells [3] and to quantify the number of internalised nanoparticles in A549 cells [4].

[1] A. Valavanidis and T. Vlachogianni, J. Pharma Reports 1 (2016), p. 105.
[2] I. Tavernaro, C. Cavelius, H. Peuschel, A. Kraegeloh, Beilstein J. Nanotechnol. 8 (2017), p. 1283.
[3] S. Schübbe, C. Schumann, C. Cavelius, M. Koch, T. Müller, A. Kraegeloh, Chem. Mater. 24 (2012), p. 914.
[4] H. Peuschel, T. Ruckelshausen, C. Cavelius, A. Kraegeloh, BioMed Res. Int. (2015), 961208.

O4-6 invited

RADIOLABELING - AN APPROPRIATE TOOL TO STUDY THE ENVIRONMENTAL FATE OF ENGINEERED NANOPARTICLES

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The study of the environmental fate of nanoparticles requires versatile tools for the detection of nanoparticulate materials in complex systems such as soil, sewage sludge or organisms within a wide range of concentration. Challenging are the environmentally relevant low concentration of nanoparticles and the presence of background concentration of the respective elements. The radiolabeling of nanoparticles offers an excellent and robust method to enable nanoparticle detection in these complex media down to the ng/L range. Even online in-situ tracing and visualization techniques are accessible to obtain spatio - temporal process information.

Depending on the nature of the nanoparticle and the process of interest different methods for the radiolabeling of nanoparticles can be applied, like the synthesis of the nanoparticles using radioactive starting materials, the binding of the radiotracer to the nanoparticles, the activation of the nanoparticles using proton irradiation, the recoil labeling utilizing the recoil of a nuclear reaction to implant a radiotracer into the nanoparticle, and the in-diffusion of radiotracers into the nanoparticles.

For our recent studies we produced [^{44}Ti]TiO_2, [^{45}Ti]TiO_2, [^{48}V]TiO_2, [^{64}Cu]CuS, [^{64}Cu]SiO_2, [^{75}Se]CdSe/[^{65}Zn]ZnS, [^{105}Ag]Ag, [^{110m}Ag]Ag, [^{124}I]CNTs, [^{125}I]CNTs, [^{131}I]CNTs, [^{7}Be]MWCNT, [^{139}Ce]CeO_2 and [^{194}Au]Pt nanoparticles. Due to the choice of the used radionuclide (half-life, decay-mode) and the activity concentration it was possible to enable different detection methods and time scales for the investigations. All these methods go along with a careful characterization of the radiolabeled nanoparticles in respect of the radiolabeling stability and nanoparticle properties.

The radiolabeled nanoparticles have been successfully used in comprehensive environmental studies, like release studies, environmental mobility studies, fate studies in waste water treatment and plant uptake studies.

O4-7

SINGLE CELL LEVEL QUANTIFICATION OF NANOPARTICLE-CELL INTERACTIONS USING MASS CYTOMETRY

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How to study nanoparticle-cell interactions is the key question that puzzles researchers in the fields of nanomedicine as well as in nanotoxicology. In nanotoxicology, the amount of nanoparticles internalized by the cells or bound to the external surfaces of cells determines the toxic profile of those particles. In medical applications, cellular uptake and binding of medically effective nanoparticles decides their efficacy.

Despite the importance of understanding the extent and mode of nanoparticle-cell interactions, these processes are underinvestigated, mainly due to the lack of suitable user-friendly methodologies. Inductively coupled plasma mass spectrometry is a well-established method to resolve cell-associated (metal) NPs in bulk cell populations, however such analysis at single cell level remains a challenge. Here we used mass cytometry, a technique that combines single cell analysis and time-of-flight mass spectrometry, to quantitatively analyze extra- and intracellular silver (Ag) in individual Ag NP exposed human T-lymphocytes. The results revealed significant population heterogeneity: e.g., in lymphocytes exposed to 3 μg of 30 nm branched polyethylene imine coated Ag NPs/mL the extracellularly bound Ag varied from 79 to 560 fg and cellular uptake from 17 to 121 fg. Similar heterogeneity was observed in cells exposed to various doses of Ag NPs with other sizes and surface coatings, demonstrating the importance of single cell analysis when studying NP-cell interactions. Although mass cytometry has some shortcomings such as inability to analyze potential transformation or dissolution of NPs in cells, we consider this a promising new method for quantitative assessment of cell-NP interaction at single cell level.

O4-8

CHARACTERIZATION OF UNLABELED NANOMATERIALS IN COMPLEX ENVIRONMENTS

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Our highlight for nanoparticle characterization is the CytoViva Darkfield Hyperspecral Microscope. This allows the easy visualization and spectral characterization of a wide range of nanoscale materials, like liposomes, metallic nanoparticles, carbon nanotubes, viruses or bacteria in any transparent or translucent complex environments. The instrument helps also to spectrally define the toxic effect of the nanomaterials on bacteria, cells, tissues or small organisms.

The enhanced darkfield illumination optics of the microscope has a much higher contrast and a documented 10 times better signal-to-noise ratio, when compared to standard darkfield optics [1].

The microscope is equipped with either a VNIR (visible near infrared) or a SWIR (short wave infrared) transmission diffraction grating spectrograph, having a spectral resolution of 2 nm and a spectral range of either 400 nm to 1000 nm (VNIR) or 900 nm to 1700 nm (SWIR). These spectrographs can detect the pure reflection spectra of all image pixels. The collected spectra can be stored in spectral libraries and used for quantitative spectral analysis of the unique nanostructures, or for localization of the nanostructures in other samples or tissue.

If a simultaneous detection of fluorescent and non-fluorescent sample elements is required, we also offer a dual mode fluorescent module. For long-term studies with living cells, a compatible environmental chamber with a closed bath design, temperature control, perfusion and gassing, is offered.

For the nanoparticle size and distribution analysis, we offer a compact, easy to use instrument, the NanoLab 3D. This DLS (dynamic light scattering) instrument has the new modulated 3D cross-correlation technology integrated. By using this technology, the instrument can suppress fully the multiple scattering arising in concentrated samples and in this way guarantees the reliability of the measurements even in turbid samples with high concentrations.

Another application area in which NanoLab 3D delivers impressive results is DLS Microrheology. This technique is used to measure the viscoelastic properties of the samples, by accurately determining both the elastic and viscous moduli.

The NanoLab 3D system can measure the samples at a sample volume between 50 µL and 2 mL, in a temperature range of 4 – 85 °C and at a particle size range of 0.3 nm – 10 µm in diameter.

[1] P Zhang, S Park and SH Kang, Bull. Korean Chem. Soc., 36 (2015), p. 1172 - 1177.

SESSION 5

Neurotoxicity

O5-1 invited

AIR POLLUTION, MICROGLIA, AND THE LUNG-BRAIN AXIS

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Urban air pollution exposure has recently been linked to increased risk of several central nervous system (CNS) diseases and conditions, including cognitive decline and Alzheimer's disease (AD). The mechanisms mediating these effects are poorly understood. Recent findings indicate that the brain's innate immune cells, microglia, detect and respond to inhaled pollutants, where pulmonary damage may signal to the brain through circulating factors (the Lung-Brain Axis). Here, we will reveal the role of damage associated molecular patterns (DAMPs) in the microglial response to diesel exhaust particles, discuss the effects of circulating factors in the 3x-TG murine AD model, and explore how aging may impact this process. These findings provide insight into the mechanisms underlying how air pollution may activate microglia and deleteriously impact central nervous system health.

O5-2

INVESTIGATION OF NEUROTOXIC EFFECTS OF MANGANESE NANOPARTICLES IN 3D NEURAL MICROTISSUE

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Welding fumes are a complex mixture of various compounds including nanoparticles (NPs) of metallic oxide (e.g. manganese oxide or dioxide). Especially manganese (Mn) containing particles (Mn_xO_x) are thought to be associated with the neurotoxicity caused by this essential element. When bypassing the homeostatic regulation in the gastrointestinal tract (GT), as seen in welders exposed to Mn via inhalation, neurobehavioral deficits might occur [1]. After the deposition of manganese NPs in the respiratory tract Mn can be directly translocated to the brain via the olfactory system [2] or enter the blood steam from the alveolar region where approximately 14% of the total Mn mass is soluble and bioavailable [3]. Via these routes Mn can enter the brain where it accumulated predominantly in the basal ganglia (BG) as well as frontal cortical areas. Recently, a PBPK model for Mn has been developed and combined with categorical regression modeling [4] concluding that a concentration of 0.55 µg/g Mn in the BG is associated with an increase of 10% of risk for neurobehavioral deficits. The underlying mechanism causing these behavioral deficits is not completely understood but various neural cell types and neurotransmitters seem to be involved [5]. Therefore, we used (a) the *in vivo* concentration from the PBPK model and (b) three-dimensional neural microtissues (MTs) derived from rat cortex preparations (PND1-2; consisting of neurons, astrocytes, and microglia) as a suitable *in vitro* model. Based on the average MT volume we calculated that 5.3 ng of Mn/MT is equivalent to the human brain concentration. We incubated the MTs to MnO_2 nanoparticles (diameter 50-80nm) and the soluble salt $MnCl_2$ in concentrations ranging from 50-500 µM with 135 µM being equivalent to the *in vivo* concentration. Cytotoxicity (ATP assay), the activation of microglia and astrocytes and neuronal microstructures (immunocytochemistry), was measured after 24 and 48 hours. Preliminary results showed that neither MnO_2 nor $MnCl_2$ were cytotoxic in the selected concentrations. The analyses of the first biological replicate indicate some activation of microglia after 48h exposure to $MnCl_2$ but not MnO_2 in the 135 µM condition. These preliminary results need to be confirmed in further biological replicates. Moreover, the translocation of the MnO_2 nanoparticles into the microtissue and the different cells need to be confirmed by transmission electron microscopy. If the 135 µM concentration indeed affects the microstructure of the neural microtissue or induces neuroinflammation we will use Ca^{2+} imaging to investigate if lower concentrations compromise the response of neurons in the MT to the most relevant neurotransmitters glutamate and gamma-aminobutyric acid (GABA). The final results of these experiments can be used (a) to understand the mechanism of Mn containing NPs in more details and (b) to identify endpoints that allow the extrapolation from *in vitro* to *in vivo* [6].

[1]Meyer-Baron, M et al., *Neurotoxicology* 36 (2013), 1-9.
[2]Elder, A et al., *Environ Health Perspect.* 114 (2006), 1172-1178.
[3]Ellingsen, DG et al., *Environ Sci Process Impacts* 15 (2013), 357-365.
[4]Ramoju, AP et al., *Neurotoxicology* 58 (2017), 226-237.
[5]Fitsanakis, VA et al., *Neurochem Int.* 48 (2006), 426-433.
[6]The experiments were partly funded by the Leibniz Association (LRA “Nanosafety”)

O5-3

DEVELOPMENT OF AN *IN VITRO* MODEL OF NANONEUROTOXICITY USING HUMAN 3D NEURAL TISSUES

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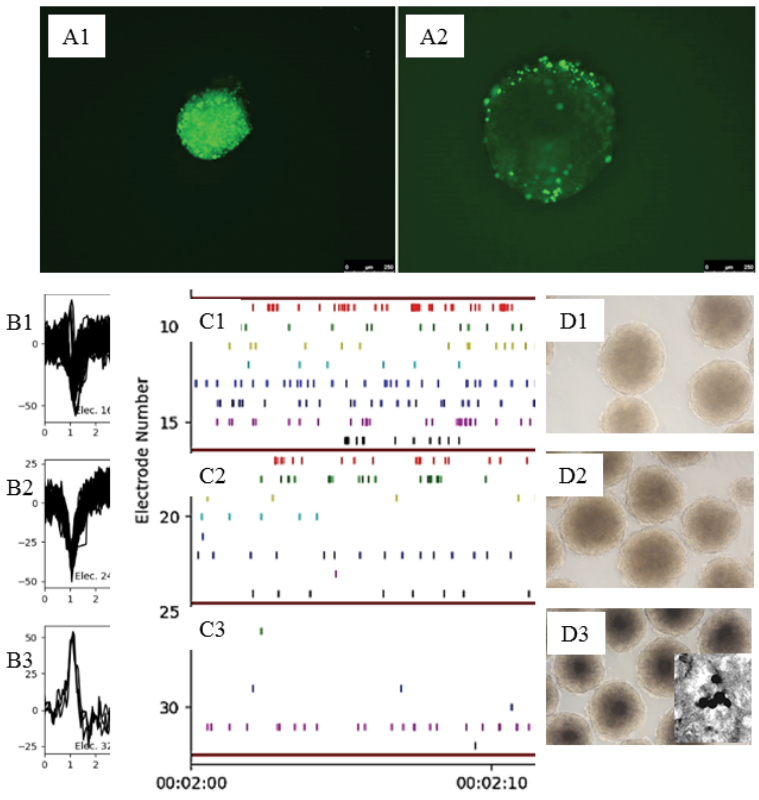
Given that many of the neurobehavioral/neurodegenerative diseases may be of environmental origin, further studies on the potential neurotoxic effects of NanoParticles (NPs), including the understanding the induction of various mechanisms leading to neuroinflammation and neurodegeneration associated with NPs are urgently needed. To answer this need, we propose to study the biological and toxicological effects induced by NPs in the Central Nervous System (CNS) by investigating the effects of the NPs on neuro-glial networks that may penetrate through to the brain parenchyma, using human *in vitro* models derived from stem-cells.

Materials and Methods: Neural stem cells derived from induced pluripotent stem cells (MTI-GlobalStem, ThermoFisher) are used to generate heterogeneous neural tissues which comprise different types of neurons and glial cells, as confirmed by histological studies. Neurospheres (NSs) are generated by aggregation of neuroprogenitors into 24 well plates under rotation. NPs are either added during the aggregation process or after the formation of the NSs. Au-NPs or Silica-FITC nanoparticles were tested in a first series of experiments. Fluorescent microscopy and transmission Electron Microscopy (TEM) were used to visualize the NPs within the nervous parenchyma. Electrophysiological recording of the activities of the neural networks were performed to assess the functionality of the neural tissue when exposed to the NPs.

Results (Figure 1): Preliminary results show as expected, that inclusion of Silica-FITC NPs (200nm; 10 µg/ml) during the aggregation process are distributed within the entire NSs (A1 after 4 days) while when added after the formation of the NSs, they remain located in the out layers of the tissue (A2 after 4 days). In another series of experiments, Au-NP (50nm) were added during the NSs formation (D1 control, D2 0.5 ppm, D3 5ppm and TEM insert). Electrophysiological recordings from those cultures after one month in culture show a clear decrease of the activities depending on the doses of the inclusions of NPs (Spikes: B1control, B2 0.5ppm, B3 5ppm) Timestamps (C1=control, C2=0.5ppm, C3=5ppm).



[1] The authors acknowledge funding from the NanoReg2 H2020 project, the Swiss Centre of Applied Human Toxicology (SCAHT) and the HES-SO, Jörg Huwyler UniBasel; Jerome Rose CEREGE, Iryna Nikonenko UniGe, Jerome Extermann Hepia

O5-4

A DAILY EXPOSURE OF RABBIT DAMS TO DIESEL EXHAUST NANOPARTICLES DURING GESTATION IMPAIRS THE BRAIN AND OLFACTORY DOPAMINERGIC PATHWAYS OF OFFSPRING

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Airborne pollution, especially from diesel exhaust (DE), is known to negatively affect the nervous system (NS) of exposed human populations, either through systemic or airway pathways. However, the consequences of a gestational exposure to DE on the NS remain poorly explored. Therefore, an experimental model of gestational exposure of rabbit dams (a human hemodichorial placentation model) to DE nanoparticles that mimics conditions of exposure in human urban areas was developed to study the short- and long-term effects of such exposure on the developing NS. Pregnant does were exposed nose-only to clean air or to diluted (1mg/m3) filtered DE from gestational day 3 (GD3) to GD27, 2h/d, 5d/w [1]. At GD28, the presence of nanosize particles (20-48nm) was observed in the olfactory sensory neurons and the glomerular layer of the olfactory bulb (OB), along with cellular and axonal hypertrophy. Concomitant bulbar monoaminergic homeostasis disturbances, especially affecting the dopaminergic system, were also observed [2]. However, the neurotransmission pathways potentially affected by this gestational exposure and their long-term consequences have yet to be further explored.

To further investigate the effects of DE exposure with a focus on the dopaminergic system, the OB and brain of GD28 (8 controls; 8 exposed) and adult (8 controls; 10 exposed) rabbits were collected. The monoaminergic pathway was analyzed using immunohistochemistry and chromatography analysis in order to assess the anatomical and functional continuum between the olfactory system and other central structures of the brain at these two states.

At GD28, the histological analysis of the OB showed an increase in the TH-labeling intensity per cell in exposed fetuses without any increase in the number of dopaminergic neurons. At the adult stage, OB of exposed animals exhibited higher levels of dopamine and its metabolites (DOPAC and HVA). Within the brain of the same rabbits, the cytochrome oxidase activity, a marker of energetic metabolism, and the TH-labeling intensity were increased in the ventral tegmental area (VTA), a key area which is implicated in the reward circuitry of the brain, whereas both markers remained unchanged in the dopaminergic pars compacta of the substantia nigra which plays a role in the regulation of the fine motor control.

All these findings suggest that the imbalance in the dopaminergic system observed in the olfactory bulb of the exposed fetuses at the end of the gestation seems to persist in adulthood, and is associated to alteration in more central structures. Because of the known anatomical and functional continuum between the olfactory system and the rest of the brain, and the importance of dopamine homeostasis in the plasticity of neural circuits, such alterations could participate to disturbances in higher integrative structures, with possible long-term behavioral consequences. [3]

[1] Valentino et al., Part Fibre Toxicol (2016), 13:39.
[2] Bernal-Meléndez et al., Toxicology Letters 259S (2016), S201-S202.
[3] Financial support: ANSES & ADEME (PNR-EST 2014-1-190).

O5-5

EFFECTS OF NANOPARTICLES IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

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There is growing concern that long-term exposure to ambient ultrafine particles may contribute to the development and progression of age-related neurodegenerative diseases like Alzheimer's Disease (AD). To address this hypothesis, we have employed the transgenic 5X Familial Alzheimer's Disease (5XFAD) mouse model, with evaluation of effects on working memory and motor function as well as on hippocampal and cortical amyloid-β plaque load. We previously showed that inhalation exposure to diesel engine exhaust (DEE) causes accelerated plaque formation and motor function impairment in these mice. DEE represents a major source of ultrafine particles in urban environments, thus suggesting that exposure to manufactured nanoparticles may also contribute to AD pathogenesis. However, in long-term exposure studies with CeO2 and SiO2 nanoparticles (by inhalation or oral exposure), we did not observe significant aggravation of AD-like features in the 5xFAD mouse model. The observed differences can be explained by contrasting physicochemical properties of the nanosize particles as well as the dose and route of exposure. Moreover, the findings from the DEE inhalation study warrant further investigation on the specific contributions of the particulate versus non-particulate components of this pollutant mixture.

The work leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° NMP4-LA-2013-310451 and the German Federal Ministry of Education and Research (BMBF/InnoSysTox-Verbund 031L0020A).

▶ SESSION 6

Nanomaterials: Effects and Mechanisms

O6-1 invited

THE EFFECT OF GOLD NANOPARTICLES IN ASTHMA MOUSE MODEL

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Abstract: Nanoparticles are increasingly used also for medical therapies. The effect of nanoparticles in healthy and asthmatic subjects is still under investigation. Here, we investigate the effect, uptake and distribution of nanoparticles in BALB/c ovalbumin (OVA) asthma mouse model. Dispersions of polyethylene-glycol-coated (PEGylated) and citrate/tannic-acid-coated (citrated) 5 nm gold nanoparticles are given intranasally to asthma and control groups. Allergic airway inflammation and airway resistance are measured. Nanoparticle uptake into extrapulmonary organs is quantified by inductively coupled plasma mass spectrometry. The asthmatic precondition increases nanoparticle uptake. Moreover, systemic uptake is higher for PEGylated gold nanoparticles compared to citrated nanoparticles. Nanoparticles inhibit both inflammatory infiltrates and airway hyperreactivity, especially citrated gold nanoparticles. Citrated and PEGylated NP-treatment in allergic asthma mice significantly prevent a strong raise of the macrophage population. Looking at the macrophages more precisely we found out that both kinds of NP does not make any shift in M1 and M2 macrophages polarization. Gold nanoparticles may have antiinflammatory effects in asthmatic mice. Asthmatic condition increases systemic uptake of gold nanoparticles. Consequent adverse effects have to be considered when designing and testing nanoparticle.

O6-2

THE IMPACT OF CUO AND TIO_2 NANOPARTICLES ON GENE EXPRESSION FOLLOWING AIR-LIQUID INTERFACE EXPOSURE

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The increasing use of nanomaterials in different industries consolidates the importance of nanotoxicological research. Up to now, most *in vitro* toxicity studies of airborne nanoparticles (NP) are performed under submerged condition. However, this exposure method is a rather artificial test system to investigate inhalation toxicity. To achieve a more realistic exposure scenario, it is possible to culture cells at an air-liquid interface (ALI). Here, cells are cultured in a transwell system, being supplied with cell culture medium from the basal side and exposed to air on the apical side. Additionally, the use of appropriate ALI exposure systems, e.g. VITROCELL® Cloud, allows the quantification of the actual particle deposition, when equipped with a quartz crystal microbalance.

Within this study, A549 cells were cultured at an ALI and exposed to CuO or TiO_2 NP in the VITROCELL® Cloud. Subsequently, cytotoxic effects were determined using the colony formation assay. Additionally, gene expression profiling via high-throughput RT-qPCR was performed [1]. CuO NP showed dose-related cytotoxic effects, while TiO_2 NP revealed no cytotoxicity. The same pattern was observed for gene expression profiling, showing no interference with gene expression by TiO_2 NP. In contrast, CuO NP exposure induced the expression of oxidative stress markers, redox-sensitive transcription factors, genes involved in DNA repair, apoptotic factors and cell cycle regulating genes.

In conclusion, a new exposure system for airborne NP was established using CuO and TiO_2 NP. CuO NP affected the expression of several genes, presumably evoked by elevated levels of ROS. However, further research is needed to confirm the transcriptional results on a functional level [2].

[1] B M Fischer, D Neumann, A L Piberger, Arch Toxicol 90 (2016), p. 2745
[2] The authors acknowledge funding from the German Federal Ministry of Education of Research, Grant Number 03XP0009.

O6-3

HOW THE PROTEIN CORONA EVOLVES ON A NANOPARTICLE

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When a pristine nanoparticle (NP) encounters a biological fluid, biomolecules spontaneously form adsorption layers around the NP, called "protein corona". The corona composition depends on the time-dependent environmental conditions and determines the NP's fate within living organisms. Understanding how the corona evolves is fundamental in nanotoxicology as well as medical applications. However, the process of corona formation is challenging due to the large number of molecules involved and to the large span of relevant time scales ranging from 100 μs, hard to probe in experiments, to hours, out of reach of all-atoms simulations. Here we combine experiments, simulations, and theory to study (i) the corona kinetics (over 10–3–103 s) and (ii) its final composition for silica NPs in a model plasma made of three blood proteins (human serum albumin, transferrin, and fibrinogen). When computer simulations [1,2] are calibrated by experimental protein–NP binding affinities measured in single-protein solutions, the theoretical model correctly reproduces competitive protein replacement as proven by independent experiments. When we change the order of administration of the three proteins, we observe a memory effect in the final corona composition that we can explain within our model. Our combined experimental and computational approach is a step toward the development of systematic prediction and control of protein–NP corona composition based on a hierarchy of equilibrium protein binding constants [3,4].

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[3]O. Vilanova, J. J. Mittag, P. M. Kelly, S. Milani, K. A. Dawson, J. O. Rädler, G. Franzese, ACS Nano 10, 10842–10850 (2016).
[4]The authors acknowledge funding from the EU FP7 NanoTransKinetics project grant NMP4- SL-2011-266737 and the Spanish MINECO Grants No. FIS2012-31025 and No. FIS2015-66879-C2-2-P.

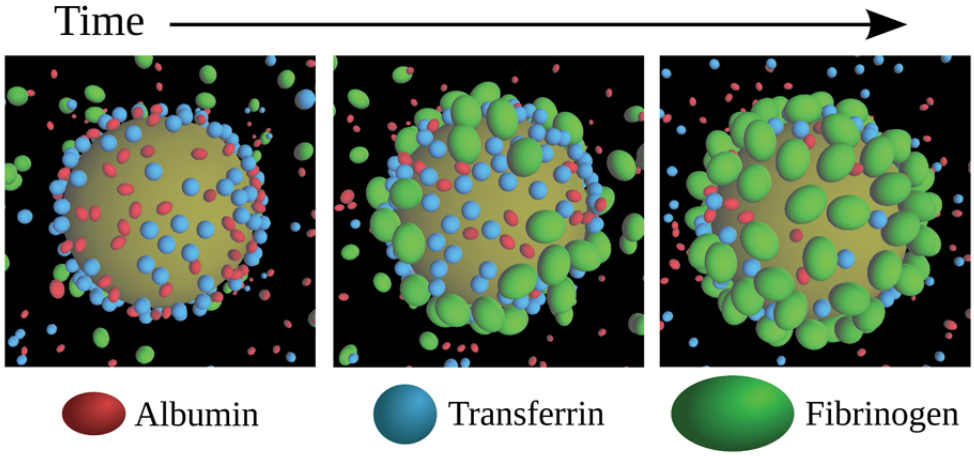

Figure 1. Kinetics of competing proteins (human serum albumin, transferrin and fibrinogen) assembling onto a silica NP of 100nm diameter. Simulation snapshots at three different times: 1st when introducing albumin; 2nd when adding transferrin; 3rd when adding fibrinogen.

O6-4

LOW-DOSE CARBON BLACK NANOPARTICLES EXPOSURE OF THE LUNG DOES NOT AGGRAVATE ALLERGIC AIRWAY INFLAMMATION IN MICE

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Asthma is a multifactorial disease defined by airway hyperresponsiveness (AHR) and inflammation. It is associated with increased mucus secretion, epithelial injury, hyperplasia of smooth muscle cells, and deposition of excess extracellular matrix. The additional irritation of the "inflamed" epithelium with inhaled nanoparticles is supposed to aggravate the asthmatic response and, at the worst, to induce an asthmatic attack.
We tested whether Carbon Black nanoparticles (CBNPs) aggravate an ovalbumin-induced allergic airway inflammation in mice. We used Printex®90 (P90) as a reference particle and P90 coated on the surface with Benzo[a]pyren (P90-BaP) or 9-Nitroanthracen (P90-9NA). Additionally, we analyzed a soot from acetylene combustion (AS) that exhibits a mixture of polycyclic aromatic hydrocarbons (AS-PAH) on the surface. Wild-type mice were sensitized to ovalbumin (OVA) and challenged with an OVA aerosol on three consecutive days. The CBNPs were applied by oropharyngeal aspiration (70 µl, 100 ng/µl) prior to the third challenge and 24 hours later.
We analyzed ex vivo the cilia-driven particle transport, cell death and mucus release in the trachea, and the mRNA expression of markers for PAH metabolism (Cyp1a1, Cyp1b1), oxidative stress (Gr, Gpx3) and the mucin Muc5ac in the tracheal epithelium and distal airways. Additionally, the amount of immune cells and TH2-cytokine levels in BAL fluids, and the AHR were analyzed.
All CBNPs were recently shown to exhibit similar hydrodynamic diameter and zeta-potential in suspension. The particle characteristics were previously described [1].
In mice with acute allergic airway inflammation, we showed that the CBNP exposure influenced neither the AHR, nor BAL cytokine levels or infiltration of leukocytes into the airways. In the trachea, the particle transport speed and the number of necrotic epithelial cells were similar to the results found in OVA-control group. Only P90 decreased the ciliary beat frequency possibly by enhanced mucus release, which was indicated by increased Muc5ac mRNA expression compared to the OVA-control group. None of the CBNP species did affect the mRNA expression of Cyp1a1, Cyp1b1, Gr and Gpx-3 in the tracheal epithelium. In distal airways, mRNA expression of Cyp1a1, Cyp1b1, Gr and Gpx-3 were slightly increased by P90-BAP and AS-PAH compared to the OVA-control group.
Our results demonstrate that the CBNP (independently of surface modification) at low concentration did not aggravate the allergic airway inflammation. Only P90 reduced ciliary beat frequency and increased mucin mRNA possibly reducing mucus clearance. [2]

[1] K Lindner, M Ströbele, S Schlick, S Webering, A Jenckel, J Kopf, O Danov, K Sewald, C Buj, O Creutzenberg, T Tillmann, G Pohlmann, H Ernst, C Ziemann, G Hüttmann, H Heine, H Bockhorn, T Hansen, P König, H Fehrenbach (2017), *Particle and Fibre Toxicology*, 14:8.
[2] The support within the nanoCOLT consortium by grant of the BMBF is acknowledged with thanks.

O6-5

UNDERSTANDING NANOSAFETY – THE IMPORTANCE OF ASSAY PERFORMANCE *IN VITRO*

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Nanotechnology is regarded as the enabling technology of the 21st century. However, compared to the numerous inventions made during the last decades only a small number of nano-enabled products finally made their way to the market. There are several reasons why these innovative approaches got stuck in translation; one key factor being uncertainties in the safety assessment of these new materials. Numerous examples exist where nanomaterials have deceived scientists by generating false positive or false negative results in *in vitro* studies [1-8]. The following parameters are important to prevent such false results and assure reliable *in vitro* safety assessment. (1) Material characterization: How well do you know the nanomaterial you are working with? How reproducible is the synthesis process? Are batch-to-batch variations to be expected? How to assess those? (2) Interference reactions with the different assay components. (3) Dosimetry: sedimentation and diffusion phenomena of particulate materials make it difficult to determine the delivered dose that is relevant for a given biological response. (4) Benchmarking and general assay performance: In comparison to other – known – (nano)materials: how strong is the observed effect? Did you think about the necessary control samples? Are all process control measurements within the specified and expected range? These issues add additional complexity to the well-known challenges of *in vitro – in vivo* comparability and finally animal-to-human predictability.

On the other hand safe by design approaches stand and fall with fast feedback loops from the material scientist (=designer) to the biologist and vice versa; precluding time consuming animal studies, longing for fast *in vitro* solutions. Especially in early developmental stages of a new, smart (nano)material the safety of a multitude of technically suitable candidates has to be assessed. Potential adverse biological effects need to be "designed out" to select for the most promising material; not only in terms of technical efficacy but also in terms of safety. Therefore fast *in vitro* tools/methods are needed. However, there is more to it than speed. Reliable, robust and reproducible (the alternative 3Rs*)* results are key to success.

My presentation will focus on research highlights from an ongoing Swiss project (NanoScreen.ch) tackling exactly the nano-related *in vitro* challenges detailed above. I will present illustrative examples of how we get to know our "simple" *in vitro* systems and why this is so important. How we elucidate sources of variability and which challenges we faced when trying to correlate material properties and biological effects. [9]

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[9] The authors acknowledge funding from the NanoScreen Materials challenge co-funded by the Competence Centre for Materials Science and Technology (CCMX).

O6-6

EXPOSURE TO NANOPARTICLES SPECIFICALLY EXTENDS NEUTROPHILIC LIFE SPAN: A TOXICOLOGICALLY RELEVANT ENDPOINT FOR NANOPARTICLE SAFETY

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Inhaled nanoparticles have been described to induce neutrophilic lung inflammation. The effects of these particles during an ongoing inflammation by interaction with the neutrophils themselves however are not well understood. Neutrophil apoptosis is considered to be a major regulator of neutrophil driven inflammatory reactions. In the current study we aimed to investigate the effects of nanoparticles on neutrophilic life span and the impact of these effects on lung inflammation in vivo.
For this purpose, mechanistic ex vivo studies with blood neutrophils from volunteers but also from COPD patients investigating neutrophil apoptosis and signalling events were performed. Additionally, lung inflammation was induced by intratracheal application of nanoparticles in Fisher 344 rats in order to correlate neutrophil apoptosis rates with the strength of inflammatory parameters in lung lavages (BAL). As we earlier described a new group of compounds to interfere with nanoparticle-induced signalling, we also investigated whether these substances (compatible solutes) have preventive effects on the anti-apoptotic signalling in neutrophils [1].

Exposure of blood neutrophils to carbon nanoparticles resulted in a significant reduction of the naturally occurring apoptosis rates [2]. This effect was mediated by the activation of Akt and, subsequently, of Mcl-1. Further mechanistic experiments indicate that induction of oxidative stress and changes in membrane structures are responsible for these nanoparticle-specific adverse outcomes. Interestingly, the prevention of the activation of these signalling events by the compatible solute ectoine resulted in a very effective restoration of the natural apoptosis rate. The in vivo relevance of the approach was demonstrated (i) in animal experiments by significantly reduced neutrophil inflammation after ectoine intervention which correlated with restored neutrophil apoptosis rates in the lung and (ii) in humans by reduced inflammatory sputum parameters after daily inhalation of ectoine for 28 days [3].

The data identify the reduction of natural apoptosis of neutrophilic granulocytes as a mechanism of the pro-inflammatory effect of nanoparticles. Moreover, as compatible solutes are well tolerated by humans, they may be used therapeutically for the treatment of chronic neutrophilic inflammation like in COPD.

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POSTER SESSION 1

P1-1

INTRINSIC AND CELL-BASED OXIDATIVE PROPERTIES OF NANOMATERIALS AND ASSOCIATED OXIDATIVE STRESS RESPONSES DETERMINED BY MULTIPLE ASSAYS

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The ability of nanomaterials (NMs) to generate oxidants/reactive oxygen species (ROS), often referred to as Oxidative Potential (OP), is a promising metric to predict the NM-toxic potency. The evaluation and testing of this hypothesis were a central aim of the nanOxiMet project. We investigated a panel of 16 NMs in different suspensions (water & cell media) regarding their OP by Electron Paramagnetic Resonance spectroscopy (EPR), dithiothreitol, 2',7'-Dichlorofluorescein-acetate (DCF-DA) and antioxidant depletion assay. Cell-based OP and oxidative stress was analysed using similar approaches, i.e. fluorescence spectroscopy, EPR and antioxidant depletion. Associated oxidative stress responses were addressed according to the 3-Tier approach in macrophage and epithelial cell lines by evaluation of mRNA expression of Nrf2-regulated genes, mRNA/protein expression of inflammatory genes and analysis of (oxidative) DNA damage, lipid peroxidation and protein oxidation. When comparing the intrinsic OP assays complementary results were found whereby two principle groups of ROS-detection approaches could be specified: (1) assays sensitive to (transition)metals (e.g. Cu, Ni) and (2) assays sensitive to materials triggering electron transfer (e.g. Ag CB). The most suitable method to determine (intra)cellular ROS formation was the DCFH-DA assay. Among the oxidative stress responses HO-1, glutathione depletion, Fpg-comet and cell viability (WST) assay were identified as most suitable. NMs may generate oxidative stress via two principal pathways, i.e. due to their intrinsic OP and upon their interaction with cells. Based on our findings several NMs caused marked oxidative stress responses while having low/negligible intrinsic OP, whereas other NMs with a marked OP only triggered minimal oxidative stress responses in cells. In conclusion intrinsic OP assays are useful tools to assess for the potential toxicity of NMs. However, cell-based OP/ROS analyses and oxidative stress responses should be included at least to minimal extent, to reduce misclassification. [1]

[1] The work has received funding from the German Federal Ministry of Education and Research (BMBF/NanOxiMet 03X0128).

P1-2

A VERSATILE PRIMARY NANOPARTICLE AEROSOL GENERATOR FOR NANOSAFETY STUDIES: INSTANTANEOUS CLOUDS AND CONTINUOUS STREAMS

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A versatile system is designed to generate nanoparticulate aerosol streams with stable concentration and particle size distribution. The device was divided into a compressed air reservoir and a nanopowder container. The operational methodology consisted in storing a dried and filtered air in a 40-cm^3 stainless steel reservoir under controlled pressure. Dried nanopowders were placed into a stainless steel column, with a 1.2-mm diameter reduction in the far end and a 50-µm grid on the bottom end. All nanoparticle aerosols were safely released into a state-of-the-art nanoparticle dispersion and exposure chamber in a nearly particle-free environment [1]. The compressed air was instantaneously released impelling the nanopowder solid through column tip. Aeroxide® TiO_2 (Evonik, Germany), ZnO (Sigma-Aldrich, USA), CuO (Nanologica, Sweden) and Multi-walled carbon nanotubes (MWCNT; Colorobbia, Italy) were successfully aerosolized.

Aerosol and offline electron microscopy tests showed the production of stable nanoparticulate streams. The air expansion in the secondary chamber and further emission using a valve produced a long-lasting aerosol plume with tunable nanoparticle concentration and narrow particle size distribution over several hours (Figure 1). Aerosol particle concentrations and median particle sizes depended on the pressure difference, being larger as the compressed air stream was increased. Particle concentration and size distribution in the nanoscale range was nearly independent on the mass of aerosolized powders.

Finally, the proposed device is a powerful tool for generating both instantaneous clouds and continuous streams of nanoparticles with wide applications in nanotoxicology and nanosafety studies [2,3].

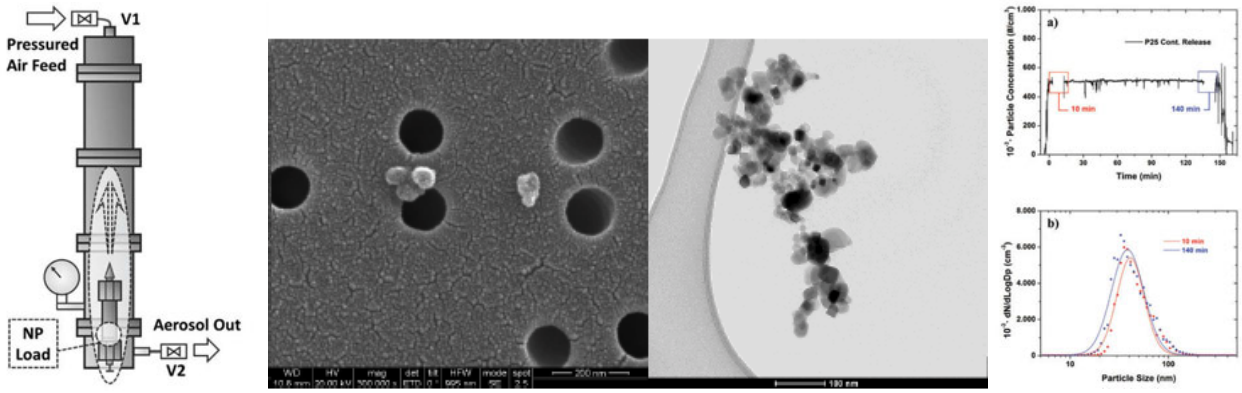

Figure 1. (a) Scheme of the continuous nanoparticle aerosol generator; (b) TEM images of TiO_2 P25 collected in the aerosol; (c) Particle concentration and size distributions of TiO_2 P25 aerosols

[1] A Clemente, MP Lobera, F Balas and J Santamaria, J. Hazard. Mater. 280 (2014), p. 226.
[2] Patent accession number P201730055
[3] The authors acknowledge funding from the EU FP7 project "NanoValid" (Grant no. 263147). Spanish MINECO is acknowledged through contracts RYC-2011-07641 and JCI-2012-13421.

P1-3

INFLUENCE OF SIZE AND SURFACE COATING ON SILVER AND GOLD NANOPARTICLES UPTAKE BY *GAMMARUS FOSSARUM*

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The increasing production and use of silver (AgNPs) and gold nanoparticles (AuNPs) will inevitably lead to their release in aquatic environments where they represent a potential threat to organisms. Therefore, there is still a need of a better understanding of the mechanisms underlying the potential toxicity of AgNPs and AuNPs. Being an important component of the aquatic macroinvertebrate assemblage, *Gammarus fossarum* will certainly be exposed to AgNPs and AuNPs if they reach the water courses and was selected as model organism for this study.

This study evaluated the acute effects of three different sizes of AgNPs and AuNPs (20, 40 and 80 nm), either stabilized with citrate (CIT) or coated with polyethylene glycol (PEG), on adult male *G. fossarum*. The tested concentrations ranged from 0.5 to 50 $\mu g.L^{-1}$.

Size distribution of AgNPs and AuNPs in exposure medium showed a higher stability of PEG-NPs while CIT-NPs aggregated. Zeta potential ranged from -20 to -17mV for CIT-NPs while it was in the range of -8 to 0mV for PEG-NPs. These results indicate a higher electrostatic repulsion of CIT-NPs compared to PEG-NPs. Ag and Au bioaccumulation revealed a significant surface-coating dependent bioaccumulation with CIT-NPs leading to a higher uptake of Ag and Au by *G. fossarum,* whereas no size-dependent effects were observed*.* Additionally, tissue distribution of Ag and Au evaluated with NanoSIMS50 revealed the presence of silver on the cuticle of the gills, while gold was present in the gut area of *G. fossarum.* The Cytoviva® dark field hyperspectral imaging allowed the the observation of the absorption of AgNPs within the tissue of the gills and the presence of AuNPs within the gut of *G. fossarum*.

These results show the influence of surface coating on the uptake and tissue distribution of AgNPs and AuNPs. The adsorption and absorption of AgNPs by the gills and the carapace might impact the respiratory, ventilation and locomotor activity of *G. fossarum* which could result in harmful effects at the population and the community level. The presence of AuNPs in the gut might disrupt the digestion processes and thus the functional role of *G. fossarum* in aquatic ecosystems. [1]

[1] This work was supported by National Research Fund, Luxembourg (AFR-PhD-9229040). The authors are thankful to S. Contal and M. Fossépré for technical support, C. Guignard for chemical analyses, E. Lentzen and A. Chauvière for NanoSIMS analyses.

P1-4

MORPHEUS – A MULTIPARAMETRIC PLATFORM FOR SAFETY TESTING OF NANOPARTICLES

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Synthetic nanoparticles (NPs) are nowadays used in numerous products in technical fields and biomedicine. An evaluation of the hazardous potential of NPs at an early stage will limit reservations in public opinion as well as provide standards for preclinical development of nanoparticles. For this purpose, a multiparametric and integrated platform for testing of NPs based on a 3D liver tissue model will be developed that include NP structure and properties, liver viability, liver functionality and morphology.

The liver, the main organ of toxic side effects, serves as a tissue model. HepG2, a liver carcinoma cell line, is used to form 3D liver microtissues by hanging drop method. The spheroids, which are harvested three days after cell seeding, have a size of about 300-400 µm. After NP exposure the cellular influence of NPs is investigated by several liver markers for vitality and function, such as the analysis of composition of cellular bile acids or the expression and activity of drug metabolizing Cyp450 enzymes, which provide a safety profile of NPs. Fluorescence microscopy is used for morphological screening of 3D liver microtissues and subcellular location of the NPs. Cell imaging showed that 50 µg/ml Atto647N-labelled silica NPs are localized only at the border, not in the middle of the spheroid. NPs penetrate about three cell layers into the spheroid (Fig. 1).

The combination of classical and new liver markers with innovative 3D cell culture technology, microscopy and PCR-based analysis of the gene expression pattern of cells in an integrated test platform will provide an important contribution to NP risk assessment.

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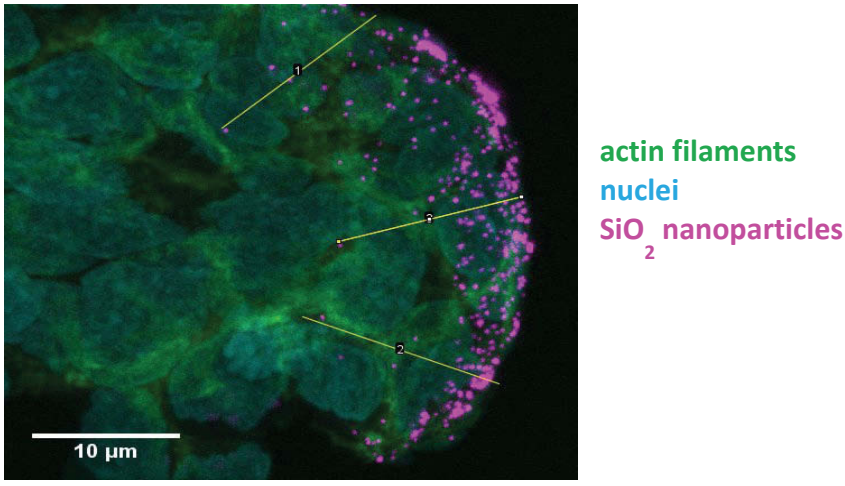

Figure 1. Fluorescence microscopy image of HepG2 cell spheroid exposed to ATTO-labelled SiO_2 nanoparticles for 24h.

P1-5

HOW SOURCE INFORMATION INFLUENCES LAY PEOPLE'S INTERPRETATION OF A SCIENTIFIC CONFLICT CONCERNING NANOSAFETY

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To make informed decisions on scientific topics, lay people often need to rely on information given by experts. However, if the positions of experts stand in conflict with each other, laypeople lack the ability to resolve these conflicts on the basis of their own knowledge. Instead, they need alternative strategies to resolve them, for instance, by using source information. Past research on this topic shows that lay people have differentiated assumptions on the cause of scientific conflicts, which include the expertise and/or benevolence of different sources [1]. In our study, we wanted to examine such effects when reading about a conflicting nanotechnology issue.

Two opposing positions on the topic of "risks of nanoparticles in sunscreen" were presented to 113 undergraduates via an online-questionnaire. All participants were told that the two positions were taken from the websites of two different scientists. Specifically, participants were told that one position stemmed from a professor of nanoscience working at a university (baseline scientist) while the opposing position - depending on the experimental condition - was said to stem from (a) a professor of nanoscience working for a company (benevolence manipulation), (b) a junior scientist working at a university (expertise manipulation), or (c) another professor of nanoscience working at a university (control group). Participants were randomly assigned to one out of these three experimental conditions. The order of source information and position was completely counterbalanced. At the end of the study, participants had to rate what in their opinion were possible reasons for the conflict between the scientists, based on a questionnaire that addressed four types of reasons (i.e., differences in the scientists' motivations, differences in the scientists' competence, differences in the scientific process, or differences in the complexity of the topic [2]). In addition, they had to rate their agreement with the two different positions. We expected (H1) the conflict in the benevolence- /expertise-manipulation to be explained stronger by differences in the motivation/competence of the scientists, and (H2) the position of the baseline scientist to be preferred in the benevolence- and expertise-manipulation compared to the control group.

In line with H1, the results of our study showed that the conflict in the expertise-manipulation group was indeed explained stronger by differences in the competence of the scientists than it was in the control group. However, a comparable effect wasn't found for the benevolence manipulation and explanations based on the motivation of the scientists. Furthermore, in line with H2 there was an influence of source information on the agreement of positions, in that the position of the baseline scientist was preferred in the benevolence- and expertise- manipulation groups compared to the control group. These findings show the important role of source information in lay people's handling of scientific conflicts.

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P1-6

DO NANOPARTICLES PASS THE INTESTINAL BARRIER? DOSE-, SIZE-, AND FUNCTIONALIZATION DEPENDENT EFFECTS *IN VITRO* AND *EX VIVO*

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Because of the common use of nano-polystyrene (PS) in many articles of daily use, the examination of this nanomaterial is very urgent. It is possible that nanoparticles (NP) enrich in different compartments of the environment and reach the food chain [1]. Moreover, there is a risk to incorporate them directly by packed food or NP-containing kitchen devices [2]. Orally absorbed NP run through the gastrointestinal tract. It is still unclear, if PS-NP are able to cross the intestinal barrier or cause local damages of the mucosal tissue. The aim of this work was the investigation of of PS-NP with different surface functionalizations and sizes (NH_2/COOH; 80 nm/600 nm) and their interaction with intestinal models at low doses.

In vitro co-culture models of two different cell types (Caco-2 and HT29-MTX-E12), were used as a 2D- and 3D-model for the nanotoxicological studies. Based on porcine small intestine tissue an *ex vivo* model was established for transport studies. *In vitro,* toxic effects were investigated regarding metabolic activity, barrier integrity and binding behavior of the PS-NP. *Ex vivo,* the toxic effect of the NP on primary tissue was analyzed via transepithelial electrical resistance (TEER) measurements and histological staining. NP transport *in vitro* and *ex vivo* was quantified via field flow fractionation (FFF).

In vitro investigations indicated size, functionalization and dose dependent toxic effects and CLSM analysis showed adhered PS-NP on the surface of both cell types (2D) and taken up NP in the inside of co-culture spheroids (3D). FFF analysis revealed that PS-NP did not overcome the intestinal barrier *ex vivo*. However, a slightly destroyed mucosa by polystyrene NP can be observed. Even in low concentrations (< 100 µg/ ml), the NP affect the functionality of the intestinal barrier. Therefore, there is a reasonable risk for humans considering NP present in the food chain.

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P1-7

EFFECT OF PONY LAKE FULVIC ACID ON THE AGGREGATION AND DISSOLUTION OF OPPOSITELY CHARGED SURFACE-COATED SILVER NANOPARTICLES AND THEIR ECOTOXICOLOGICAL IMPACT ON *DAPHNIA MAGNA*

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The interactions between nanoparticles (NPs) and natural organic matter (NOM) have been widely investigated in many studies due to their significant role in the environmental fate of NPs [1,2]. Despite increasing efforts in the last years, still more systematic studies are needed to understand the effects of NOM and surface properties of NPs on their aging, fate, and ecotoxicity. In this study, we investigated how the colloidal stability and dissolution of oppositely charged silver nanoparticles (Ag NPs) and their ecotoxicity are dependent on the type of coating agents and the presence of NOM. As a model NOM, Pony Lake Fulvic Acid (PLFA) was selected due to its high affinity to metallic Ag and Ag^+ ions.

The presence of PLFA influenced the dissolution and ecotoxicity of the oppositely charged Ag NPs. The extent of dissolution for both positively and negatively charged Ag NPs decreased at low concentration of PLFA. In contrast, the release of dissolved Ag increased at high concentration of PLFA but only for positively charged Ag NPs. The toxicological impact of Ag NPs on *Daphnia magna* was up to 70% lower than in the absence of PLFA. However, high content of Ag in *Daphnia magna* exposed to the positively charged Ag NPs showed the possibility of accumulation and enrichment of Ag in organisms feeding on *Daphnia magna.* The type of dissolved Ag species (free Ag^+ ions or dissolved Ag complexes with PLFA) and their bioavailability were key factors influencing the ecotoxicity of Ag NPs on *Daphnia magna*. Consequently, the findings of this study demonstrate that our results can provide a sound basis for further studies to understand the mechanisms responsible for the fate and ecotoxicological impact of Ag NPs especially in the presence of NOM and possible enrichment of positively charged nanoparticles in the food web [3].

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P1-8

EFFECTS OF COLLOIDAL SILICA NANOPARTICLES ON EGFR-DEPENDENT SIGNAL TRANSDUCTION

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Engineered nanomaterials are becoming increasingly important from economic and industrial points of view. Humans can be exposed to nanomaterials through different routes, like injection or swallowing of nanoparticles in medical use or therapeutic applications. Various studies showed that interactions of nanomaterials with biological systems of human cells are determined by the physicochemical properties of the particles (element composition, morphology, surface chemistry, etc.). Whether positive or negative effects for human cells are elicited depends on the nature of nanoparticle cell interaction.

Colloidal silica nanoparticles prepared from tetraethylorthosilicate were tested for their ability to affect the signal transduction pathways of epithelial cells. Here, we investigated the response of rat epithelial cells (RLE 6TN) exposed to three different size classes of colloidal silica nanoparticles and non-colloidal control nanoparticles. Besides cytotoxicity, the molecular effects of the particles on cell growth and signal transduction were in focus. Above that, the uptake mechanisms of the silica nanoparticles and subcellular localization of the particles were investigated.

We showed that 15 nm and 25 nm colloidal silica nanoparticles exhibited an inhibitory effect on the epidermal growth factor-(EGF-) mediated cell proliferation while 80 nm or non-colloidal silica nanoparticles had no effect. Exposure to 15 nm silica nanoparticles resulted in a reduction of EGFR phosphorylation at tyrosine residue 1173. The nanoparticles significantly reduced EGFR translocation and the activation of intracellular signal kinases, particularly protein kinase B (Akt), which are associated with the activation of the EGFR. The molecular mechanisms of the anti-proliferative effect of the nanoparticles were identified at the level of interaction with the silica nanoparticles and the natural ligand EGF causing a loss of EGF-function.

Confocal microscopy indicated colocalization of the particles and EGF within vesicular cell structures. Further analyses with endocytosis inhibitors showed that the silica nanoparticles were internalized employing clathrin-dependent mechanisms. Using fluorescent markers for sub-cellular vesicles, silica nanoparticles localized in early and late endosomes. Thus, it was concluded that silica nanoparticles after internalization are transported to the lysosomes.

Our findings demonstrate a so far undescribed interaction of colloidal nanoparticles with a growth factor resulting in a reduction of cellular growth rates. These results may be of importance for medical applications in which EGF-dependent cell growth has to be suppressed.

P1-9

METHODS TO ASSESS THE EFFECTS OF NANOPARTICLES ON MUCOCILIARY CLEARANCE *EX VIVO*

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Inhaled particles and pathogens are removed from the airways by a mechanism termed mucociliary clearance. Secretory epithelial cells release mucus that captures inhaled pathogens and particles. This mucus is then removed by ciliated epithelial cells whose ciliary beating transports the mucus towards the larynx. Impairment of mucociliary clearance leads to less effective removal of infectious and toxic substances and can lead to disease.

To assess possible toxic effects of nanoparticles on mucociliary clearance, we developed a model using the explanted murine trachea.

For analysis, the trachea was explanted, the trachealis muscle was cut and the trachea was transferred to a culture dish filled with HEPES-buffered Ringer solution that was kept constant at 30°C.

Ciliary beat frequency (CBF) was determined by high speed video microscopy followed by fast Fourier analysis of grey level changes due to ciliary beating. The capacity of the epithelium to transport particles was assessed by addition of 4.5 µm polystyrene particles. The particle transport speed (PTS) was determined by video microscopy and subsequent automated tracking of individual particles. Mucus present in the preparation was detected by fluorescently labelled Ulex europaeus lectin-1 and wheat germ agglutinin and visualized by fluorescence microscopy. The same preparation was used to isolate epithelial cells for subsequent mRNA expression analysis of the airway mucin Muc5ac using quantitative RT-PCR. Chemical fixation of the tissue allowed determination of epithelial morphology by scanning electron microscopy or the detection of necrotic or apoptotic epithelial cells by staining with ethidium homodimer-1 or for activated caspase-3, respectively.

In healthy tracheae unaffected by nanoparticles, PTS correlated well with CBF. Mucus released by nanoparticles was detectable by lectin-staining. If mucus release was moderate, CBF increased to compensate for reduction of PTS but larger amounts of mucus led to an overall reduction of PTS. The mRNA expression of mucin Muc5ac did not correlate with the presence of mucus on the healthy tracheal epithelium after exposure to nanoparticles. In addition to mucus release, we also observed that nanoparticles reduced PTS by induction of epithelial cell death and accumulation of dead cells on the epithelial surface that impaired transport.

In summary, we present a set of *ex vivo* methods that provides analysis of parameters that modulate mucociliary transport and can be influenced by nanoparticles.

P1-10

SELF-CLEANING NANO-EXPOSURE CHAMBER TO VALIDATE THE ENVIRONMENTAL AND OCCUPATIONAL IMPACT OF AIRBORNE NANOPARTICLES

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Engineered and manufactured materials at the nanoscale, NOAAs (nano-objects, aggregates and agglomerates) have the potential to improve quality of life, providing benefits to the environment, and enabling societal advances. At the same time, their increasing production, commercialization and use in various consumer or industrial applications will increase the exposure of humans and ecosystems to these new materials and will therefore require robust control of the associated potential risks.

To tackle the issue, an airtight and self-cleaning nano-exposure chamber is designed to validate environmental and occupational scenarios [1]. The release of hazardous nanoparticulate matter in accidental situations was simulated in a specially designed 13-m^3 stainless steel airtight chamber, which allowed the dispersion analysis of airborne matter in a practically particle-free environment (less than 2 #/cm^3) and in presence of background atmospheric aerosols. ENPs aerosols are introduced in the chamber with a controllable total population and size of well characterized ENPs under exposure-causing activities at production plants and laboratories employing nanomaterials, as transfer of ENPs solids or combustion of nanocomposites. They are assessed using SMPS sampling and also by TEM, SEM analysis. Additionally the evolution of the degree of agglomeration of ENPs aerosols under different environmental conditions (temperature, humidity, aerosol concentration) was also studied. The development of this new testing environment allows: (1) the generation of ENPs aerosols in laboratory conditions, avoiding the human exposure during assays; and (2) the effective control of test environmental conditions allowing a complete investigation of the ENPs emission. The results obtained suppose significant advances in the analysis of ENP dispersion in different conditions and the analysis of environmental and occupational impact in nanotechnology scenarios in a controlled way and perform measurements, which for a real-life situation are usually difficult to arrange and expensive.

Figure 1. Image of the airtight and self-cleaning nano-exposure chamber

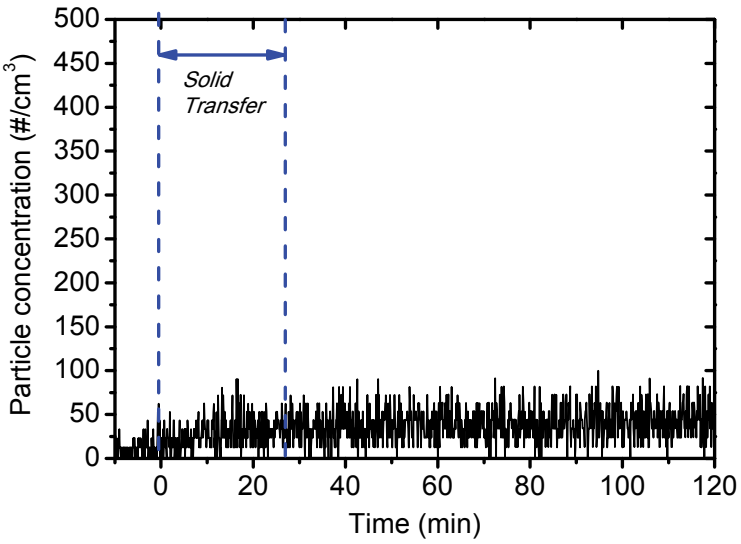

Figure 2. Particle concentration of SiO_2-NP based aerosols in a practically particle-free environment.

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P1-11

CONCEPT BASED SEARCH IN THE FIELD OF NANOTOXICOLOGY

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Risk assessment of current and future nanomaterials is facing many different challenges, one of them being the continuously increasing amount of nanotoxicology related publications. Information retrieval is therefore a first and very important step for any researcher or regulator dealing with issues of risk assessment of nanomaterials. Traditional approaches to extract information and to maintain an overview over such a rich information resource are query engines, which allow for the formulation of so-called "free text" queries typically consisting of single words or small phrases. The returned and ranked documents will contain the search terms.

However, when querying a literature database, such as PubMed [1] or even the DaNa knowledge base [2], we typically want to extract documents, which contain not only single words or phrases but rather one or more concepts. A major challenge for this approach is to use words or phrases describing the concept we are looking for. Then again, this can lead to a number of problems. Many words have more than one meaning, like the term "Paris" that for example represents the name of several cities, the son of King Priamos in the Greek mythology or even the name of a plant. Likewise, the actual appearance of the used words within the text is uncertain since synonyms ("Paris" vs. "French capital"), or hypernyms ("Paris" vs. "capital" or "city") can be used instead.

To overcome these problems, new approaches [3, 4] try to extract more semantic information from the text and shift the search from the word-level to the concept-level. This is done by an additional disambiguation step, which identifies the concepts, which are described in the documents. Using this approach, the user does no longer use words that are expected to appear within the documents, but instead uses words to describe the concepts one is looking for. With the help of an appropriate auto-completion system [5], this can be done easily and also improves the quality of search-result considerably.

Such an approach will be developed within the next phase of the DaNa2.0 project not only to support and facilitate ongoing activities but also to help moving forward the field of nano risk assessment and information provision for the public. DaNa2.0 is a national project funded by the German Federal Ministry of Education and Research (FKZ 03X0131).

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P1-12

INVESTIGATION OF HUMAN PULMONARY MUCUS USING HIGH RESOLUTION MICROSCOPY

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Mucus is a viscous secretion, consisting of ~ 95% water and other components, such as mucins, lipids, proteins and DNA, which functions as a barrier to protect the underlying cellular surfaces. The mucin glycoproteins are responsible for the gel-like structure of mucus [1, 2]. Understanding of the formation and the relevant parameters for the structure of mucus is of special interest regarding particle penetration and the effects of nanoparticles on the mucus barrier and underlying cells. The network properties which were found by cryoSEM [3, 4] are directly relevant for its size filtering barrier properties [5].

The aim of this study was to visualize the structure of human mucus under native conditions using Confocal Laser Scanning Microscope (CLSM) and Stimulated Emission Depletion Microscope (STED), which provides an improved, nano-scale resolution. Gel forming glycoproteins were labeled with fluorescent wheat germ agglutinin (which binds to N-acetylneuraminic acid (sialic acid) and N-acetylglucosaminyl residues) and specifically labeled with fluorescent antibodies directed to specific mucins. We observed that the latter labeling method provided a better visualization of the mucus structure. In addition to mucins, also other components of the mucus structure were complementarily labeled, in order to improve the understanding of mucus structural composition. Drying and dilution during the handling process were considered as important factors that could affect the native state of mucus. Our results also show a porous “foam-like” structure [6] in the native state morphologically similar to the images obtained by cryoSEM. This successful visualization of the structure of native mucus reveals pore-like structures ranging from some nanometers to several micrometers.

In the next steps, this investigation will be extended by correlating these data to other relevant microscopic methods. This study improves the knowledge about the structure of native mucus and can be used as an investigation method in nanosafety studies with respect to penetration and interaction of mucus with nanoparticles and nanotechnological inhalation pharmaceuticals.

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P1-13

LUNG TOXICITY TESTING OF AEROSOLS FOR *IN-VITRO* STUDIES THE AIR-LIQUID INTERFACE WITH INTEGRATED DOSE MONITORING

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The state of the art *in vitro* studies on aerosol health effects is based on submerged exposure of agglomerated particulate matter, suspended in culture medium. However this method does not represent the actual process in the human lung [1]. It may change the properties of the investigated particles and moreover neglects the gas phase including their interactions with particles and cells.

Exposure at the Air-Liquid Interface (ALI) avoids these disadvantages, but requires a comprehensive system to guarantee reproducible conditions. Therefore KIT and VITROCELL Systems developed a fully automated ALI exposure station [2]. The exposure station offers a complete measurement system for parallel exposure of up to 24 human lung cell cultures towards gases, nanoparticles and complex mixtures such as combustion aerosols. The aerosol flow, temperature, and humidity are adjusted to the conditions resembling the human lung. An internal negative control using humidified synthetic air is also implemented and by electrostatic particle deposition the particle dose per time can be increased. The deposited particle dose is monitored online using a quartz crystal microbalance [3].

Several measurement campaigns were successfully performed using the Exposure Stations for ALI experiments: aerosols from different biomass heaters and aerosolised industrial nanoparticles were characterised with respect to cytotoxicity, ROS-formation, and inflammatory effects using established methods as well as omics methods [4,5].

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P1-14

DEVELOPMENT OF A NANOPARTICLE AEROSOL EXPOSURE SYSTEM FOR ELUCIDATING INTERACTIONS BETWEEN NANOPARTICLES AND AIRWAY EPITHELIAL IMMUNITY IN THE FRUIT FLY *DROSOPHILA MELANOGASTER*

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With the development of nanotechnologies the risk for exposure of humans to engineered nanoparticles (NPs) has considerably increased over the years. Concomitantly, indications for adverse health effects have emerged. Inhalation of NPs, as one important route of exposure, has been associated e.g. with the exacerbation of pre-existing respiratory diseases such as lung fibrosis, COPD or asthma. However, the effects are strongly dependent on the size, shape, chemical composition, surface modification or electrical properties of the particles [1]. Therefore, the existing data are very diverse and hardly comparable.

In the mammalian respiratory system, airway epithelial cells are the first cells which come into contact with airborne particles such as microorganisms, allergens or NPs. When activated, they recruit immune cells of the innate and adaptive immune response additionally to their own immune response [2]. Hence, classical animal models such as mice are not suitable to study the interaction of NPs and epithelial cells only.
The fruit fly *Drosophila melanogaster,* however, which has been shown to be an excellent model organism to investigate airway epithelial immunity, allows a more focused view on the interplay of NPs and airway epithelial cells *in vivo*. The fly's respiratory tract consists of only a single layered epithelium without contribution of immune cells, interstitial or smooth muscle cells [3]. Moreover, a plethora of available genetic tools facilitates investigations on single aspects of immune signalling or potential genetic targets.

The first part of this project requires the development of a NP exposure system with regard to the special challenges that come along with exposing the small airways of larvae (openings: 6-12 µm) or adult flies. We therefore use an electrospray aerosol generator which enables us to provide monodisperse NPs in a dry state and at controlled concentrations.
This system will subsequently be used in the second part of the project for investigating the effects of silica NP exposure on the epithelial immune response of *Drosophila* such as production of antimicrobial peptides. For the characterization of the epithelial immune response we use transgenic flies in which the activation of immune signalling pathways or single pathway components is coupled to the synthesis of GFP and can therefore be detected with the help of fluorescence microscopy. The authors acknowledge funding and support by the Leibniz Association [4].

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P1-15

HOT SPOT RELEASE MAPPING OF NANOMATERIALS

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In the early phase of the development of nanomaterials especially of the next functionalized generation, we are confronted with the Collingridge dilemma between far reaching design options on the one hand and a very limited availability of reliable knowledge about expectable impacts.

Based on possible available information the poster will present a preliminary visual exposure assessment method. The tool integrates expert knowledge and literature data about release points and release quantities and extends the modeling approach to the entire life cycle of the nanoapplication.

Interviews of expert answers to questionnaires and literature survey build the basis for a detailed visualization of the life cycle stages of nanoapplications as well as for the weighting of release potentials. Based on this hot spots maps are created in which the environmental releases potentials are marked with an arrow and the weight of the flows corresponds with the thickness of the lines.

Thus the relevant nanospecific release potential can be easily identified along the life cycle stages of the nanoproduct and this knowledge may be used in the process of a precautionary product development and product use optimization. Some examples of hot spots release maps developed in the SUN project will be presented.

POSTER SESSION 2

P2-1

CERTAIN ENGINEERED NANOMATERIALS INDUCE AMYLOID AGGREGATION AND NEURODEGENERATION PROCESSES IN THE NEMATODE *CAENORHABDITIS ELEGANS*

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Exponentially growing nanotechnology causes the release of nanoparticles (NPs) into the environment and increases the probability of human exposure. The invertebrate model animal *Caenorhabditis* (*C.*) *elegans* is abundant in the soil ecosystem and serves as a tool for the investigation of nanotoxicological pathways.

The worm's transparency enables localization of NPs in single cells and correlation of particle distribution with organ function. We show that certain nanomaterials (NMs) such as silica NPs induce amyloid protein aggregation as well as neurodegenerative processes [1]. Mass spectrometry analysis identified a silica NP-induced aggregome network that contains predominantly proteins involved in protein homeostasis (proteostasis). Disturbed proteostasis leads to an inhibition of serotonergic neurotransmission that in turn perturbs the egg laying behavior of *C. elegans*. We suggest that silica NPs promote a cascade of events from amyloid formation and neurodegeneration to disturbed neurotransmission. Compounds preventing amyloid fibrillation interrupt these neurodegenerative processes [2]. In order to monitor neurodegenerative NP-biointeractions over the entire life span we cultivated adult hermaphrodite *C. elegans* in 96-well plate microhabitats that mimic the worm's microbe-rich boom habitat. Cultivating worms in liquid media in 96-well microtiter plates enabled analysis of nanomaterial effects in a life span-resolved manner and identification of age-dependent vulnerabilities of specific eNMs. We show that nano silica and nano silver specifically target middle-aged nematodes by promoting neuromuscular locomotion defects. NP-induced uncoordinated locomotion represents premature aging, since it is normally observed in old *C. elegans*. By comparing multiple endpoints such as life span, age-resolved behavior and neurotoxicity we demonstrate that Ag NPs induce concentration-dependent toxicity compared to ZnO NPs or CeO_2 NPs [3, 4].

Since major physiological pathways including neurotransmission pathways are conserved between worms and humans, stress responses can be compared between both species. The relevance of neurodegeneration enhanced by nano silica and nano silver in worms for human health will be discussed.

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P2-2

SAFE-DESIGNING OF CUO NANOPARTICLES

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The safe implementation of nanotechnology requires nanomaterial hazard assessment in accordance with the material physicochemical properties that trigger injury response at the nano/bio interface. While CuO nanoparticles are widely used in the sensors, catalytic and semiconductor industries, the dissolution properties of these materials play a major role in their hazard generation. We hypothesized that Fe doped CuO might offer a safer by design approach by decreasing the rate of Cu^{2+} dissolution [1]. To test this hypothesis, the library of pure and/or Fe doped CuO was synthesized using flame spray pyrolysis. The physicochemical properties of the materials were extracted using advanced analytical techniques including XRD and BET, Raman spectroscopy, HRTEM, and EELS. The structural investigation of pure CuO showed a significant reduction in the apical Cu-O bond length while simultaneously increasing the planar bond length (Jahn-Teller distortion). Cyclic voltammetry was used to establish the surface interaction propensities of the biological biological redox species when these particles enter a living organism. Hazard screening was performed in tissue culture cell lines and zebrafish embryos to discern the change in the hazardous effects of doped *vs*. non-doped particles. The increased levels of doping showed progressive decrease in cytotoxicity in BEAS-2B and THP-1 cells, as well as an incremental decrease in the rate of hatching interference in zebrafish embryos. In summary, a safe-by-design strategy was demonstrated for the toxic CuO particles *via* Fe doping and is proposed for safe implementation of these particles in the environment.

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P2-3

THE LEIBNIZ NETWORK NANO - NANOTECHNOLOGY IN THE LEIBNIZ-ASSOCIATION

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The Leibniz Association is one of the four major science associations in Germany. It connects more than 90 independent research institutions covering a wide range of scientific topics ranging from natural-, engineering-, life- and environmental sciences to economics, spatial- and social sciences to the humanities.
The Leibniz Network Nano is a network of meanwhile 16 institutes of the Leibniz Association. The network is coordinated by an office 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.
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, nano sensors and nano optics 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 nano scale form another focus area, in which electron microscopy has a particular important role. Finally an important focus exists in the field of nano medicine, nano biology and nano safety.
This poster briefly describes the activities in nanotechnology within the Leibniz Association as reflected in the Leibniz Network Nano.

P2-4

A NEW INTEGRATED MEASUREMENT APPROACH TO SUPPORT THE IMPLEMENTATION OF THE COMMISSION RECOMMENDATION FOR THE DEFINITION OF NANOMATERIAL

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The EC recommendation for the definition of nanomaterial [2011/696/EU] requires the quantitative size determination of constituent particles in samples down to 1 nm. Accordingly, a material is a nanomaterial if 50 % or more of the particles are in the size range 1-100 nm. The fact that engineered nanomaterials already exist in many industrial and consumer products challenges the development of measurement methods to reliably identify, characterize and quantify their occurrence as substance and in various matrices.

The EU FP7 NanoDefine project [www.nanodefine.eu] has addressed this challenge by developing a robust, readily implementable and cost-effective measurement strategy to obtain quantitative particle size distributions and to distinguish between nano and non-nano materials according to the EU definition. Based on a comprehensive evaluation of existing methodologies and intra- and inter-lab comparisons, validated measurement methods and instrument calibration procedures have been established to reliably measure the size of particles within 1-100 nm, and beyond, including different shapes, coatings and chemical compositions in industrial materials and consumer products. Case studies prove their applicability for various sectors, including food, pigments and cosmetics.

Main outcome is the establishment of an integrated tiered approach including rapid screening (tier 1) and confirmatory methods (tier 2), and a user manual to guide end-users, such as manufacturers, in selecting appropriate methods. Another main product is the “NanoDefiner” e-Tool allowing the standardised / semi-automated selection of appropriate methods for material classification according to the EU definition. Results also contribute to standardization efforts, such as CEN TC 352 or ISO TC 229. [1]

[1] The project has received funding from the European Union’s Seventh Programme for research, technological development and demonstration under grant agreement No 604347

P2-5

SAFE-BY-DESIGN: A CHANCE FOR SMART AND SAFE NANOINNOVATIONS

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Due to the novel combination of size, structure, and physical/chemical properties (e.g., optical, electrical, catalytic, magnetic, adhesion properties), nanoscience and nanomaterials can permit remarkable technological advances and innovations in many industrial sectors [1]. However, the nanoscale opens the doors to new or different potential risks that are still not fully explored. Moreover, regulation of nanomaterials seems to be unable to keep up with the high speed of innovation [2,3]. To address the needs of nano-manufacturing companies, we propose a sustainable safety concept with a balanced approach between design for manufacturing and design for safety [4]. Presently, the model is used in ongoing H2020 pilot line projects synergistically (e.g., Hi-Response, INSPIRED, R2R Biofluidics, Smart-4-Fabry), and encompasses six main pillars (Fig. 1):

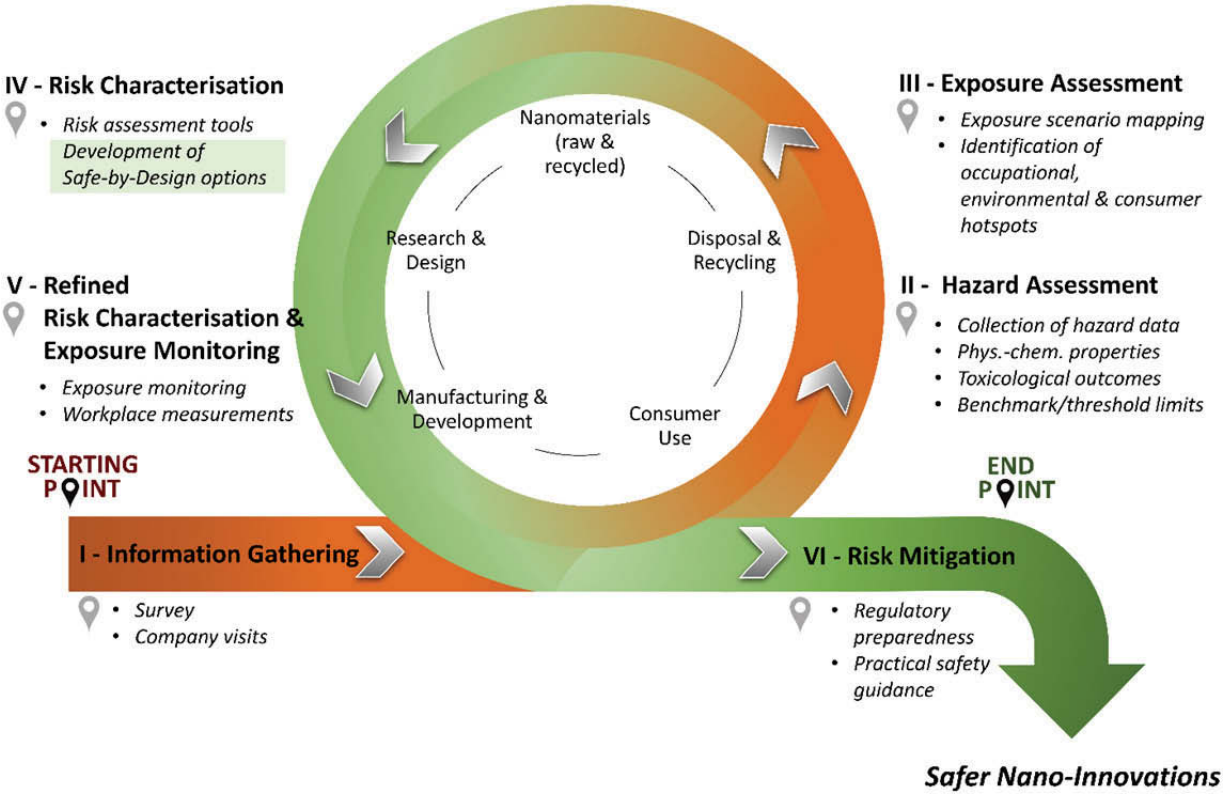

Figure 1. Illustration of the proposed safety strategy

The prosed approach provides a lifecycle hazard-, exposure- and risk profile for a given nanomaterial (e.g., classification of which materials/process operation pose greater risk, where these risks occur in the lifecycle, and the impact of these risks on society). Use of the concept cannot prove safety or guarantee absolute safety. However, implementation of Safe-by Design early in the development process can form an important cornerstone in making products and materials fit for a circular economy and offers a chance for smart innovation.

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P2-6

MULTI-WALLED CARBON NANOTUBES: INTERACTION WITH LUNG EPITHELIAL CELLS

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Carbon nanotubes (CNTs) are cylinders of single or multiple graphene layers rolled to form a tube. Their mechanical, electrical, and thermal properties make them interesting for many applications, such as electrochemical devices, fillers, sensors, or drug delivery vehicles. Although not all CNTs exist as straight or rigid fibers, their fiber-like structure has led to the suggestion that CNTs behave similarly to mineral fibers or synthetic vitreous fibers, which according to the fiber paradigm, can induce inflammation, fibrosis, lung cancer, and mesothelioma as well as direct and indirect genotoxicity [1]. After inhalative uptake of CNTs, they come into direct contact with type II alveolar epithelial cells, the most frequent cell type in the deep lung. These cells interact with macrophages and fibroblasts, and yet have not received much attention in the context of CNT-induced effects. Besides their main function of lung surfactant production, alveolar type II cells also play an important role in restoring the epithelial barrier after damage. Therefore, not only cytotoxic effects, but also inflammatory and fibrotic processes have to be studied [2].

A detailed understanding of mechanisms induced by nanomaterials requires both: information on the type of interaction between the materials and cellular structures as well as elucidation of biologically relevant responses induced by the presence of the nanomaterials. Therefore, aim of this work was to assess the interactions between well-characterized, long and short multi-walled CNTs with A549 cells as a model for human alveolar type II cells. The state of the CNTs, their interactions with the cytoplasmic membrane, as well as their internalization were in focus. In addition, cellular responses were monitored. Carbon nanofibers served as a reference material.

Cytotoxicity assays revealed a slightly increased metabolic activity after CNT exposition, but no impaired membrane integrity. Low vacuum scanning electron microscopy (SEM) was used to visualize the CNT interaction site with the cytoplasmic membrane. CNT tips seemed to cause membrane invaginations but no obvious membrane damage. A correlative microscopy approach combining SEM and confocal laser scanning microscopy (CLSM) proved the latter to be a reliable method to confirm CNT internalization as well as to track their intracellular fate. Furthermore, NF-κB translocation to the nucleus in consequence of CNT exposition (3 µg/ml, 24 h) was disproved by immunofluorescence (IF) staining. A next step towards a better understanding of CNT cell interactions that have been observed with SEM, is to visualize the contact area in detail.

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P2-7

APPLYING SAFER-BY-DESIGN CONCEPT WITH POLYMERIC NANOBIOMATERIALS FOR DRUG DELIVERY

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Polymeric nanobiomaterials are expected to have much potential in medicine, such as: to increase efficacy and safety by targeting drug delivery and to deliver drug to specific tissues or malignant cells. However, the number of health applications based on polymeric nanobiomaterials on the market remains small [1]. This might be due to various factors: a) the unclear situation of the future regulatory requirements for efficacy and safety, and b) guidelines for testing nanomaterials which are still under development. All those challenges are hampering SMEs to further develop and exploit the potential of nanotechnology.
In this context, the GoNanoBioMat project aims to support SMEs which are either active in the field of polymeric nanobiomaterials for drug delivery or have some links to this field (e.g. producing nanoparticles or producing polymeric nanobiomaterials) or are potential entrants to this interdisciplinary field in the future. The major expected outcome of the project is guidelines to help SMEs to systematically develop safer polymeric nanobiomaterial drug delivery systems. To do so, we plan to use Safer-by-Design (SbD) approach as structural backbone. Even if SbD approach is more and more used in nanosafety research projects [2], they often do not clearly state what SbD means and lack to acknowledge the challenge to implement it in real life [3]. Therefore, we intend to create a SbD approach for polymeric nanobiomaterials for drug delivery and concretely apply it with three case studies: a) Chitosan, b) PLA, and c) PHA.

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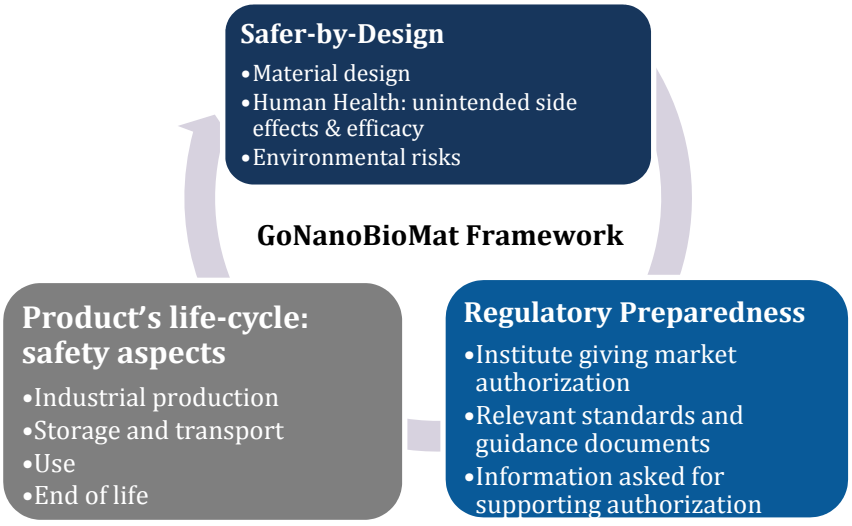

Figure 1. Framework of the GoNanoBioMat project, including SbD approach, Regulatory preparedness and safety aspects of the product's life-cycle.

P2-8

MIXTURE TOXICITY OF SILVER ION COMBINED WITH TIO_2 AND ZNO ON DAPHNIA MAGNA

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Silver (AgNPs), TiO2 and ZnO nanoparticles (NPs) are one of the most extensively used engineered nanomaterials. Due to their widespread use in industry, it can lead to increase the release of these nanoparticles into the environment. Being released into various environmental components, NPs may simultaneously interact with other NPs or dissolved ion states. We believe that the combined mixture of NPs could be exposed simultaneously to aquatic organisms. This research aimed at assessing potential synergistic toxicity of combined binary mixture of NPs in NOEC or LOEC. Evaluation of mixture toxicity is based on the concentration addition (CA) model which is the classic concept for predicting the effects of chemical mixtures with similar mechanisms of action. In order to assess their toxic effects, we conducted acute toxicity test with *Daphnia magna* from single and binary mixtures exposure. Based on their single toxic effect concentrations (ECs) values, we applied CA model to predict ECs values of mixtures and estimated the interactions between components in mixtures by model deviation ratio (MDR). In addition, we performed physicochemical characterization of NPs using SEM, UV-VIS and Zeta potential measurements. With the CA model used, the present study shows the great different mixture toxicity between the single toxicity and mixture toxicity. The level of toxicity is surely contributed by their aggregation as well as physicochemical properties. In conclusion, Toxicity levels of Ag ion combined ZnO and TiO2 revealed synergistic and ZnO and TiO2 mixtures are determined to be antagonistic in the EC_{15} and a greater aggregation of NPs in combined mixtures than in single NPs. Further studies concerning interaction of NPs in mixture need to be understand their fates in the aquatic environments.

P2-9

CARBON NANOPARTICLES INDUCE CELL CYCLE ARREST IN LUNG EPITHELIAL CELLS

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Exposure to particulate air pollution is correlated with age-associated degenerative diseases of the airways. Particularly emphysema, a main factor of chronic obstructive pulmonary disease (COPD), may be induced by inhaled environmental particles. However, the mechanistic background of this pathogenic effect is so far not well understood. Besides inflammatory reactions triggered by the particles, a direct interaction of inhaled particles with cells of the airway epithelium may cause cellular reactions responsible for these adverse health effects. In previous studies we demonstrated that carbon nanoparticles as model particles for environmental combustion-derived particles induce stress induced senescence accompanied by cell cycle arrest in lung epithelial and endothelial cells [1]. Cell cycle arrest in lung epithelial cells has been suggested to be a cause of age-associated airway diseases due to potential loss of regenerative capacity. We therefore aimed to investigate if the regenerative capacity of lung epithelial cells is impaired by carbon nanoparticles.

For this purpose, the cell cycle of lung epithelial cells repetitively exposed to low, non-cytotoxic doses of carbon nanoparticles was monitored by flow cytometry. Moreover, the histone deacetylase SIRT-1, a known cell cycle regulator and marker of lung aging, and Thioredoxin-1, which is reduced in stress-induced senescence, were analysed. Furthermore, Connexin 43 was investigated because intercellular communication is decreased in lung diseases like COPD.

Exposure of lung epithelial cells to carbon nanoparticles led to cell cycle arrest. Analyses of apoptosis rates at the level of DNA content and of caspase-3 activation demonstrated that neither programmed cell death nor necrosis are responsible for this effect. Moreover, at the protein level, significant reduction of SIRT-1 and active Connexin 43 were observed upon particle exposure. Accordingly, in rats the repetitive application of carbon nanoparticles diminished SIRT-1 and active Connexin 43 in lung tissue.

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P2-10

LIFE CYCLE THINKING OF NANOTECHNOLOGY BASED APPLICATIONS

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The EU FP7 SUN – Sustainable Nanotechnologies - project has investigated specific nanomaterials and associated products during the life cycle of the products in different life cycle assessment (LCA) case studies:

- Nano-WC-Cobalt (Tungsten Carbide-cobalt) sintered ceramics
- Nanocopper wood preservatives
- Carbon Nano Tube (CNT) in plastics
- Silicon Dioxide (SiO2) as food additive
- Nano-Titanium Dioxide (TiO2) air filter system
- Organic pigment in plastics
- Nanosilver (Ag) in textiles

Environmental impacts of the production of nanomaterials depend on the type of manufacturing process (energy demand, demand of operating supplies, yield, purification rate). In the case studies we can see a great range of factors of environmental impacts of the production of nanomaterials in comparison with microsized or conventional materials.

The poster will present examples of the results of life cycle assessments on the SUN case studies and will discuss the following questions: What is the environmental impact of the production of nanomaterials? What is the influence of these nanomaterials on the environmental impact of new (prospective) applications? Which kind of nanoapplications we need in future to realize high environmental (sustainable) benefits?

P2-11

FULL-PARTICLE NANODESCRIPTORS

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A set of novel, theoretical full-particle nanodescriptors[1] for modeling, grouping or read-across of metal oxide NPs properties and biological activity was developed based on the force-field calculation of the potential energies of whole NPs. Based on the core-shell model, 35 individual nanodescriptors were constructed solely from the nanostructure itself. These descriptors were derived from the chemical composition, potential energy, lattice energy, topology and size. We also demonstrate the relevance of these nanodescriptors over the Principal Component Analysis (PCA). The grouping provided by the PCA approach was found to be in good accordance with the experimental[2] algal growth inhibition data.

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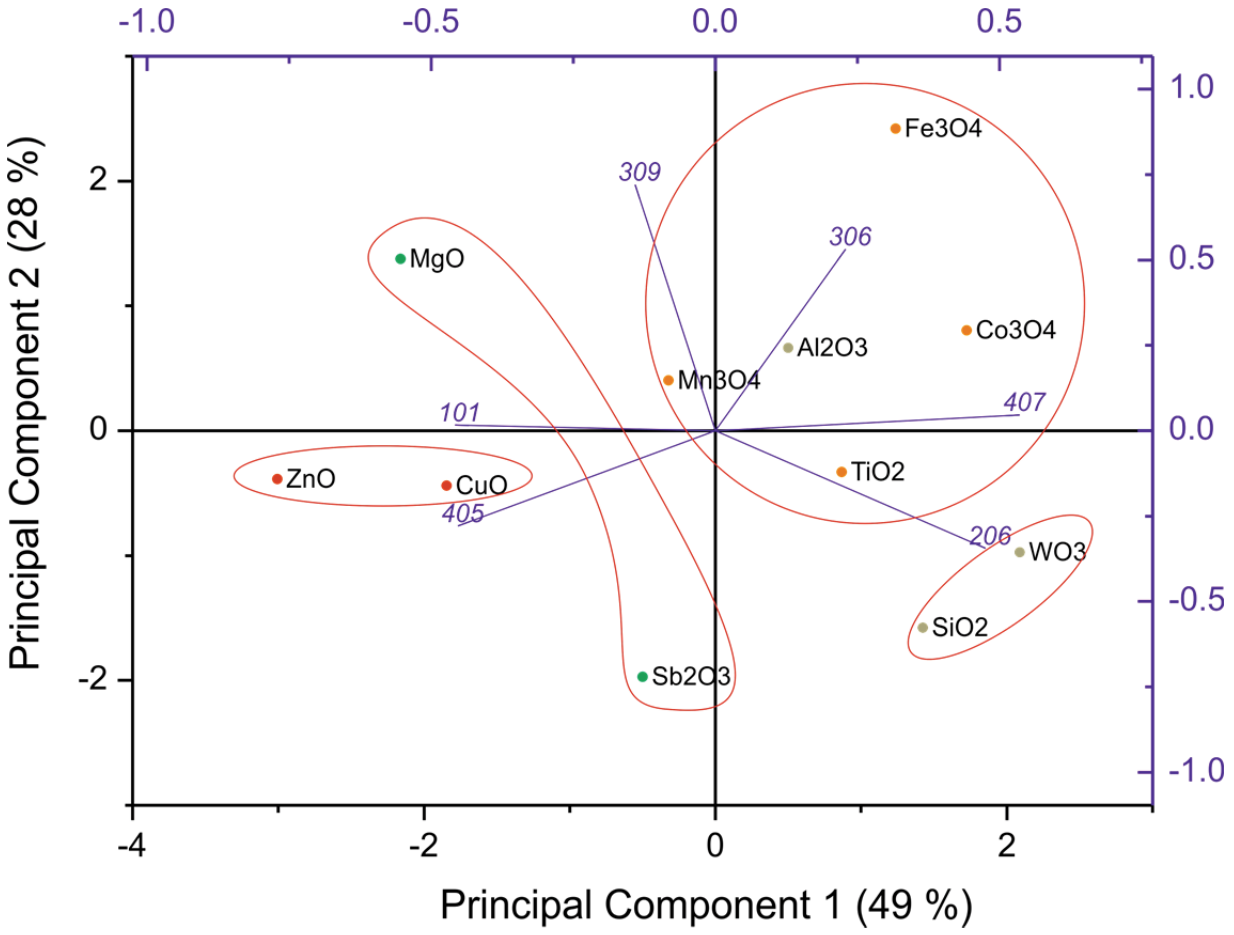

Figure 1. Two-component PCA plot for 11 oxides with 6 descriptors used in the analysis. Toxicity scale from very toxic to non-toxic: red>orange>olive>green.

P2-12

NANOPARTICLES ACTIVATE THE EPIDERMAL GROWTH FACTOR RECEPTOR VIA A NON-CANONICAL MECHANISM IN LUNG EPITHELIAL CELLS

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Poorly soluble nanoparticles are used in many consumer products. Therefore, humans might be exposed to these materials via different pathways. In order identify potential risks of modern nanomaterials we investigate pro-inflammatory pathways triggered by nanoparticles involving the activation of the epidermal growth factor receptor (EGFR).

The current study aimed to identify the initial mechanism of EGFR activation by nanoparticles compared to ligand-dependent activation. For that purpose, the effects of carbon nanoparticles on lung epithelial cells *in vitro* and *in vivo* were investigated.

EGFR activation by carbon nanoparticles led to its translocation from the lipid raft fraction of the cytoplasmic membrane to intracellular non-raft fractions. This process was accompanied by the co-localization of the structural protein caveolin-1. Receptor activation by the natural ligand EGF, however, had no impact on caveolin-1 localization, while EGFR as a feature of activation translocated into the cytoplasm. Furthermore, caveolae formation determined by oligomerization of caveolin-1 was only observed when cells were treated with carbon nanoparticles but not after EGF exposure. Blocking caveolae formation by the pharmacological inhibitor filipin III resulted in a significant reduction of the nanoparticle-specific pro-inflammatory signalling via protein kinase B (Akt). The *in vivo* relevance of these findings was corroborated in experiments comparing caveolin-1 knock out animals with their wild type littermates. In nanoparticle-exposed animals expressing caveolin-1, lung epithelial cells showed an activation of Akt, while in knock-out animals this signalling pathway was not activated. This specific effect was reflected at the level of lung inflammation. Broncho-alveolar lavages of exposed caveolin-1 knock out animals showed a significant reduction of neutrophil influx compared to exposed wild type animals.

The findings demonstrate that poorly soluble nanoparticles address a specific non-canonical mechanism of EGFR activation involving caveolae formation in epithelial cells. Our *in vivo* data demonstrate that in the system of the airways, this highly specific signalling pathway is responsible for neutrophilic lung inflammation induced by nanoparticles. [1]

[1] The authors acknowledge funding from the Deutsche Forschungsgemeinschaft (GRK 1427)

P2-13

DEVELOPING A TRUSTED ENVIRONMENT FOR SHARING KNOWLEDGE OR INFORMATION ON NANOTECHNOLOGY INNOVATIONS AS PART OF SAFE BY DESIGN

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When it comes to nanotechnology, technological innovations happen very fast, and safety governance is not keeping pace. Regulation and governance of safety are not enhanced or innovated quickly enough. This creates a burden on innovation, since the business case has been or will be adversely affected, development of inherently safe substances are cancelled or because the public calls for a halt. These developments are economically and socially undesirable and they affect the competitiveness of the European economy. Innovation governance should be able to effectively deal with complications like these.

Effective innovation is about developing new ideas into profitable business cases and going to market as fast as possible, but it is also about dealing with societal risks, related to the environment, public health and working conditions. This calls for sharing information and knowledge between actors from early phases of innovation onwards. We regard it essential for development of safe innovations that information and knowledge exchange between these worlds will be improved substantially. Trust is key to come to meaningful and acceptable exchange.

How Europe should deal with the challenges of public private knowledge exchange and uncertainty with regard to regulation, is determined by developments on innovation and by the governance of the ecosystem in which these innovations take place. Good innovation governance consists of strengthening the innovation ecosystem. This includes encouraging knowledge circulation, improving relations between the actors and providing support between demand and supply where possible.

As a part of safe by design we intend to govern this information and knowledge exchange by means of a so-called Trusted Environment: a means to exchange knowledge and information throughout the innovation process between companies, universities and research institutes (innovators) on the one side and governments (regulators) and semi-governmental organizations on the other side. This environment is considered an important aspect of good innovation governance.

For this we are developing a blueprint of the Trusted Environment. After examining existing interaction platforms, as well as perceived barriers and incentives to share knowledge and exchange views and information, a first design of the Trusted Environments has been developed. This design has been collaboratively developed with practitioners of the public and private sector from all over Europe. In 2017 and 2018 simulations with all stakeholders will be held.

The authors would like to present the first results of the design – a first conceptual prototype of the Trusted Environment. This presentation builds on the barriers and incentives for information sharing in the innovation ecosystem of nanotechnology. The current situation has been examined and preconditions have been investigated to possibly overcome the barriers that currently exist.

We believe that presenting these results could provide for a valuable addition to the discussion on safe by design, safe production and usage of nanomaterials and knowledge transfer of scientific results to support socially relevant aspects. Similarly, we are very interested in receiving feedback.

P2-14

PAMAM DENDRIMERS FOR NEURONAL APPLICATIONS: EVALUATION OF INTERNALIZATION, BIOCOMPATIBILITY AND SYNAPTIC EFFECTS

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Polyamidoamine (PAMAM) dendrimers are hyperbranched polymers which have been widely studied as drug nanocarrier systems. Despite of their promising results in different *in vitro* and *in vivo* models, important aspects about their interaction with neurons to generate safe and more specific applications for central nervous system remain unclear [1, 2].

In order to explore the effects of PAMAM dendrimers with different surface properties in relevant aspects for neuronal applications, three different PAMAM dendrimers were used, G4: fourth generation PAMAM dendrimer with unmodified positive charged surface, PFO_{25}: G4 modified in 25% of their superficial amines with folate, and $PPEG_{25}$: G4 PAMAM modified in 25% of their superficial amines with polyethylene glycol. The ability to enter to neurons and the effects in neuronal viability, Ca^{2+} physiology and synaptic activity of G4, PFO_{25} and $PPEG_{25}$ was evaluated using primary cultures of hippocampal neurons as the study model.

Confocal microscopy showed that both G4 and PFO_{25} dendrimers are similarly up-taken by neurons, being possible to observe their internalization at 2 h of incubation. However, $PPEG_{25}$ has a slow internalization which is observed for incubations longer than 6 h. Cytotoxicity evaluation showed that G4 induces a significant reduction in neuronal viability, which is attenuated by both chemical modifications, being $PPEG_{25}$ the most biocompatible of them. Moreover, G4 induced a significant increase of 8-fold in intracellular Ca^{2+} and a significant increment of 3-fold in frequency of synaptic events in patch clamp recordings. Interestingly, both PFO_{25} and $PPEG_{25}$ did not induce significant changes in total Ca^{2+} intake and synaptic activity.

Taken together, these results demonstrate that surface properties of PAMAM dendrimers are key to determine their interaction with neurons. Cationic G4 dendrimers are able to rapidly enter to neurons, but induce toxic effects and alterations in normal synaptic activity. On the other hand, functionalization with both folate and polyethylene glycol can prevent the toxic effects and synaptic alterations. However, PFO_{25} and $PPEG_{25}$ have different internalization and neuronal viability patterns, which could be explained because of the differences in size of polyethylene glycol and folate, and the subsequent different surface charge density of the generated dendrimers.

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P2-15

EFFECTS OF SIO_2 AND CEO_2 NANOPARTICLES ON THE PROTEOLYTIC PROCESSING OF THE ALZHEIMER-ASSOCIATED B-AMYLOID PRECURSOR PROTEIN

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Alzheimer´s Disease (AD) is the most common form of dementia, affecting 20-30 million people worldwide. Histopathologically AD is characterized by the presence of extracellular senile plaques and intracellular neurofibrillary tangles [1]. AD associated senile plaques are mainly consistent of the Amyloid β-protein (Aβ) which is generated by sequential proteolytic processing of the β-Amyloid precursor protein (APP) by two proteases called β- and γ-secretase [1]. The initiation and progression of AD is a complex process and the exact mechanism is not yet fully understood. Only 5% of all AD cases are due to genetic mutations while the remaining 95% of cases are of sporadic nature and the causality behind is multifactorial. Among others, environmental factors, such as metal-based nanoparticles, have recently been discussed in relation to AD causation [2].

Whether manufactured nanoparticles might play a major role in the development and progression of AD has not been determined so far. It forms a challenging topic of investigation and could provide an important contribution to nanosafety. For that reason we determined the effects of selected manufactured nanoparticles, namely CeO_2, SiO_2 and carbon black (CB) on AD related features *in vitro*. In a first attempt, 293 human embryonic kidney (HEK) 293 cells were exposed to the distinct nanoparticles and the generation of Aβ was monitored. In contrast to SiO_2 and CB exposure, treatment of HEK293 cells with manufactured CeO_2 nanoparticles led to decreased secretion of Aβ. The molecular mechanism behind the altered Aβ generation was determined by analysing the proteolytic processing of APP. Treatment with CeO_2 nanoparticles led to an altered pattern of the APP processing products which could be potentially due to an altered activity of one of the proteases involved in the proteolytic processing of APP.

These results indicate that exposure to manufactured nanoparticles, depending on their physico-chemical properties, could affect the development of AD related features.

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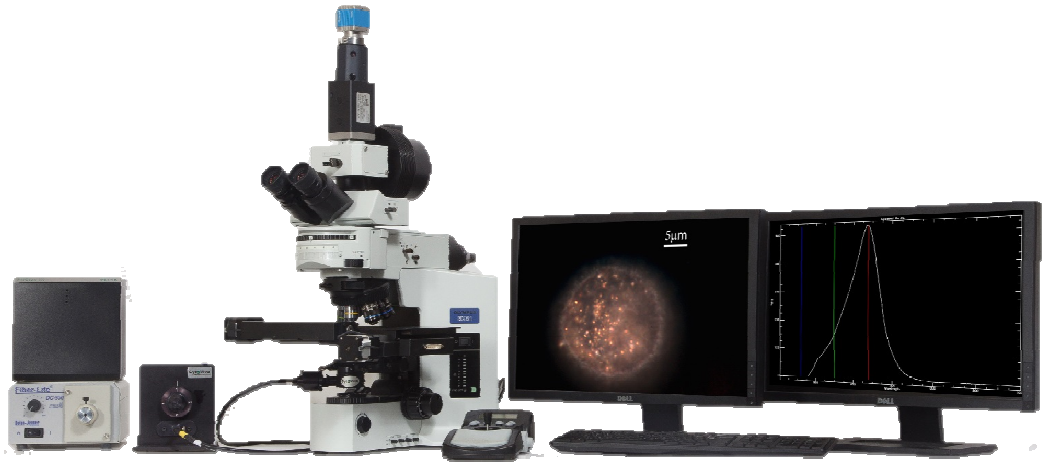

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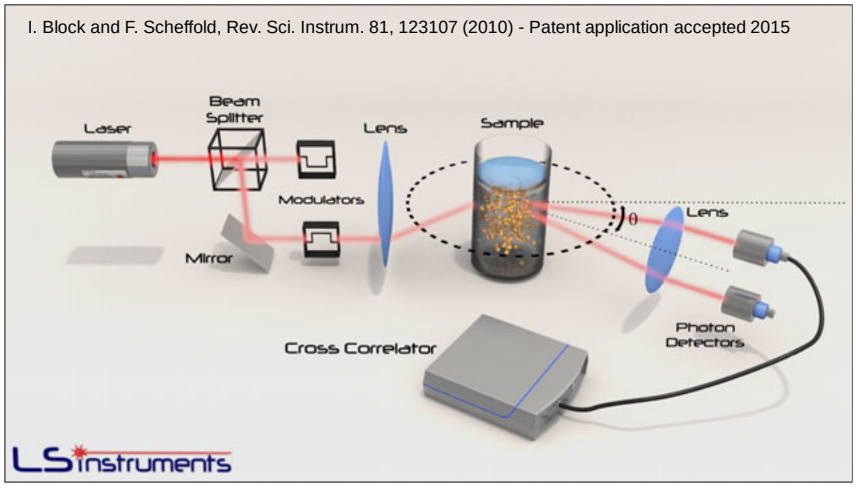

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