# **SENN**2015

International Congress on Safety of Engineered Nanoparticles and Nanotechnologies

12 - 15 April 2015
Marina Congress Center, Helsinki, Finland

## **Programme and Abstracts**

The Congress is organized by the Finnish Institute of Occupational Health (FIOH) and supported by the "NANOSOLUTIONS" EU 7th Framework Programme Project (grant agreement no 309329).

Finnish Institute of Occupational Health
Helsinki, Finland 2015

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# Dear Participants

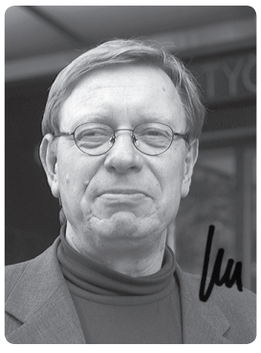
*The Nanosafety Research Centre of the Finnish Institute of Occupational Health welcomes you to the SENN2015 "International Congress on Safety of Engineered Nanoparticles and Nanotechnologies" to be held on 12 – 15 April 2015 in Helsinki, Finland.*

*The goal of the Congress is to summarize and share the latest knowledge on the safety of engineered nanomaterials and nano-related technologies. The emphasis is on producing solutions to the safety challenges related to engineered nanomaterials and nanotechnologies. Another aim is to enable commercial opportunities for the safe use of these materials and technologies.*

*The Congress will provide a forum for reporting and demonstrating findings, methods, tools and approaches to safety and health at workplaces using nanoparticles and nanotechnologies. The plenary and free communication sessions will be designed to facilitate interaction between participants and presenters.*

*We wish you a rewarding Congress experience.*

*On behalf of the SENN2015 Organizing Committee*



***Kai Savolainen**, Professor*
*Director of Nanosafety Research Centre, Finnish Institute of Occupational Health*
*Chair of the SENN2015 Organizing Committee*

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# Sunday, 12 April 2015

**9:00–18:00** **Registration and information at Scandic Grand Marina Hotel lobby, Katajanokanlaituri 7, Helsinki**

**FURTHER EDUCATION LECTURES (PARALLELS)**
Venue: Scandic Grand Marina Hotel

**10:00**

**Lecture 1A:**
**Physico-chemical characterisation of nanoparticles in suspension and embedded in biological matrices**
Erik H. Larsen, Denmark
**Room: Eliel-Selim**

**Lecture 1B:**
**Introduction to systems nanotoxicology**
Dario Greco, Finland
**Room: Compass**

**11:30** **Break**

**12:30**

**Lecture 2A:**
**Occupational exposure assessment - Measurement, modeling and control banding**
Keld A. Jensen, Denmark
**Room: Eliel-Selim**

**Lecture 2B:**
**Cardiovascular effects of nanomaterials**
Fritz Krombach, Germany
**Room: Compass**

**14:00** **Break**

**14:30**

**Lecture 3A:**
**Approaches for predictive nanotoxicology: Feasibility and challenges for risk assessment of nanomaterials**
Karin Aschberger, Italy
**Room: Eliel-Selim**

**Lecture 3B:**
**Environmental effects of synthetic nanomaterials: nanoecotoxicology**
Anne Kahru, Estonia
**Room: Compass**

**16:00** **End of further education sessions**

**18:00–20:00** **SENN2015 Get-together**

Venue: Scandic Grand Marina Hotel, Room Commodore (lobby floor)
Address: Katajanokanlaituri 7, Helsinki

# Monday, 13 April 2015

**7:30-17:30** Registration and information at Marina Congress Center, 2nd Floor, Katajanokanlaituri 6, Helsinki

Room: Fennia II
Chair: Kai Savolainen

**8:45** Congress Opening
Harri Vainio, Director General, Finnish Institute of Occupational Health, Finland

**9:00** **KEYNOTE 1:** Opportunities and challenges of carbon-based nanomaterials
Maurizio Prato, Italy

**9:30** **KEYNOTE 2:** Nanomaterials: One Viewpoint of a Materials Scientist
Olli Ikkala, Finland

**10:00** Break: Coffee/tea

**10:30–11:45** **FREE COMMUNICATION SESSIONS (PARALLELS FC1 + FC2)**

## FC1: INFLAMMATION AND GENOTOXICITY

Room: Fennia II
Chair: Hannu Norppa

**10:30** Fibre-induced gene and microRNA expression changes in human lung cells: a comparative study on MWCNTs, asbestos and glass wool
**Penny Nymark**, Peter Wijshoff, Julia Catalán, Pekka Kohonen, Hannu Norppa, Roland Grafström, Jos Kleinjans, Jacob J. Briedé

**10:45** Suitability of in vitro tests for the assessment of the toxicity of nanoparticles with surface plasmon resonance (SPR) properties
**Mary Gulumian**, Leigh-Anne Koekemoer, Kailen Boodhia, Jiya George, Natasha Sanabria, Charlene Andraos

**11:00** Can the comet assay be used reliably to detect nanoparticle-induced genotoxicity?
**Hanna L. Karlsson**, Sebastiano Di Bucchianico, Andrew R. Collins, Maria Dusinska

## FC2: MATERIALS

Room: Fennia I
Chair: Minnamari Vippola

Comparison between soft (liposomes) and hard (carbon nanotubes and graphene oxide) functionalised nanomaterials as inducers of in vivo neuroinflammation
Corinne Portioli, **Cyrill Bussy**, Mariarosa Mazza, Marina Bentivoglio, Kostas Kostarelos

Comparison of in vitro and in vivo formed protein coronas: Implication for targeting and cellular internalization
**Marilena Hadjidemetriou**, Zahraa Al-Ahmady, Kostas Kostarelos

Development of nanomaterial libraries for nanosafety studies: Polyvinylpyrrolidone (PVP) Capped Metal Oxide Nanoparticles
**S.M. Briffa**, I. Lynch, V. Trouillet, M. Bruns, D. Hapiuk, Dang, Z. Y., R.E., Palmer, N. Sano, E. Valsami-Jones

11:15 Iron Oxide Nanoparticles: Effects on cellular uptake, cytotoxicity and differentiation of rat bone marrow derived mesenchymal stem cells
**Shrestha Surakshya,** Zheng-Wei Mao, Chang-you Gao

11:30 Enzymatic -stripping' and degradation of PEGylated single-walled carbon nanotubes by neutrophils elastase and myeloperoxidase
**Kunal Bhattacharya,** Cristiano Sacchetti, Ramy El-Sayed, Andrea Fornara, Gregg P. Kotchey, James A. Gaugler, Alexander Star, Massimo Bottini, Bengt Fadeel

The role of surface functionalization in the genotoxicity of different nanomaterials in vitro
**Gerard Vales**, Satu Suhonen, Kirsi Siivola, Julia Catalán, Kai Savolainen, Hannu Norppa

Who will produce the safe nanomaterials? University teaching content in nanotechnology studies does not match job skill demands in the nanotechnology industry
Bartlomiej Szafran, Pawel Wojcik, Bartlomiej Spisak, Karen Griffin, Dorota Rutkowska-Zbik, Paula Queipo,Thomas Zadrozny, Costas Kiparissides, Olga Kammona, Frederick Ntow, Steffi Friedrichs, **Albert Duschl**

11:45–12:45 Lunch

Room: Fennia II
Chair: Robert Landsiedel

12:45 **KEYNOTE 3:** Opportunities and risks of nanomaterials – What can we learn by looking through a magnifying glass?
Barbara Rothen-Rutishauser, Switzerland

13:15 **KEYNOTE 4:** Taking stock of the current status of medical surveillance and epidemiologic research for nanomaterial workers
Paul Schulte, NIOSH, USA

13:45 **POSTER SESSION 1** (see page 46) + coffee break
Room:Nordia

15:00–16:15 **FREE COMMUNICATION SESSIONS (PARALLELS FC3 + FC4)**

**FC3: PULMONARY TOXICITY: BIOLOGICAL AND METHODOLOGICAL CONSIDERATIONS**
Room:Fennia II
Chair: Anna Shvedova

15:00 Pulmonary effects and biokinetics of nanoparticles: Interim results of the long-term inhalation study with CeO2 and BaSO4
**J. Keller**, K. Küttler, L. Ma-Hock, V. Strauss, S. Gröters, K. Wiench, W. Wohlleben, G. Oberdörster, B. van Ravenzwaay, R. Landsiedel

**FC4: EXPOSURE AND PROTECTION**
Room: Fennia I
Chair: Thomas Kuhlbusch

Detailed size and chemical characterization of ultrafine particles in workplaces and comparison to toxicity studies with engineered nanoparticles
Firdevs Ilci, **Jeremy Gernand**

15:15 **Taquann dispersion method and direct injection whole body inhalation system**
**Jun Kanno**, Yuhji Taquahashi, Atsuya Takagi, Masaki Tsuji, Koichi Morita, Yukio Ogawa, Akihiko Hirose

15:30 **Integration of micro RNA and transcriptomic sequencing profiles underlying the response of rat lung to inhaled silver nanoparticles**
**Chang Guo**, Joanna M. Seiffert, Kian Fan Chung, Rachel Smith, Timothy W. Gant, Martin O. Leonard

15:45 **Towards the prediction of nanoparticle-induced inhalation toxicity: Evaluation of an in vitro macrophage assay**
**Martin Wiemann**, Antje Vennemann, Lan Ma-Hock, Karin Wiench, Robert Landsiedel

16:00 **Inflammatory response from 52 volunteers after 75 min exposure to laser printer emissions**
**Rudolf Schierl**, Rudolf Jörres, Stefan Karrasch, Myriam Ehret, Britta Herbig, Dennis Nowak, Stefan Seeger, Jeanette Langner

**Tiered approach measurement strategies for inhalation exposure to Nanoobjects and their agglomerates and aggregates: Testing the sensitivity of decision criteria**
**Derk Brouwer**, Ruud Boessen, Cindy Bekker, Carsten Möhlmann, Delphine Bard, Rinke Klein-Entink

**Framework to forecast exposure of the next generation of nanomaterials**
**Henk Goede**, Tom Ligthart, Jessica Meyer, Simon Clavaguera, Elise Boukris, Imelda van de Voorde, Thomas Kuhlbusch, Derk Brouwer

**Mapping occupational exposure to MNMs in construction**
**Celina Vaquero**, N. Galarza, J.L. López de Ipiña, M. Jaen, R. Pina, I. Larraza, B. Hargreaves

**Process-generated nanoparticles, ignored and uncomfortable sources of workplace exposure to nanoparticles**
**Pieter Van Broekhuizen**, Rokus Renirie

16:15 Room: Fennia II

**PLENARY: INTERACTIVE DISCUSSION**
**"Do systems biology approaches have a role in the new paradigm of safety assessment" (Title TBC)**
Moderator: Bengt Fadeel, Sweden
Invited commentary: Agnieszka Kinsner-Ovaskainen, EC

17:00 End of day

19:00–20:30 **Helsinki City Reception**
Venue: Helsinki City Hall
Address: Pohjoisesplanadi 11 - 13, Helsinki

# Tuesday, 14 April 2015

8:30–17:30 Registration and information at Marina Congress Center

Room: Fennia II
Chair: Mary Gulumian

9:00 **KEYNOTE 5:** Collection and characterization of released nanomaterials from nano-enabled products: NANOSOLUTIONS Case Studies
Socorro Vasquez-Campos, Leitat Technological Centre, Spain

9:30 **KEYNOTE 6:** Genotoxicity of nanomaterials: challenges and nanospecificity
Hannu Norppa, FIOH, Finland

10:00 Break: Coffee/tea

10:30–11:45 **ORAL FREE COMMUNICATION SESSIONS (PARALLELS FC5 + FC6)**

**FC5: ORGAN TOXICITY AND BIOKINETICS**
Room: Fennia II
Chair: Ulla Vogel

10:30 Changes in cholesterol homeostasis and acute phase response following pulmonary MWCNT exposure links MWCNT to risk of cardiovascular disease
**S.S. Poulsen**, A.T. Saber, A. Mortensen, J. Szarek, D. Wu, A. Williams, O. Andersen, N.R. Jacobsen, C.L. Yauk, H. Wallin, S. Halappanavar, U. Vogel

10:45 Nickel and nickel oxide nanoparticles cause distinct genotoxic effects in human lung cells
**Sebastiano Di Bucchianico**, Anda R. Gliga, Bengt Fadeel, Hanna L. Karlsson

11:00 In vitro toxicity evaluation of indium tin oxide (ITO) nanoparticles on human lung epithelial A549 cells
**Yosuke Tabei**, Akinari Sonoda, Yoshihiro Nakajima, Vasudevanpillai Biju, Yasukazu Yoshida, Masanori Horie

11:15 In vivo toxic effects of uncoated zinc oxide nanoparticles
**Julia Catalán**, Marit Ilves, Antti J. Koivisto, Satu Suhonen, Kirsi Siivola, Maciej Stepnik, Jolanta Gromadzinska, Joanna Roszak, Anna Smok-Pieniazek, Esa Vanhala, Henrik Wolff, Harri Alenius, Kai Savolainen, Hannu Norppa

**FC6: RELEASE DETECTION**
Room: Fennia I
Chair: Arto Säämänen

Release of nanosized pigments from paints under wet use phase solicitations
**Brice Fiorentino**, Delphine Boutry, Sylvie Motellier, Jean-François Damlencourt

Workplace measurements in the facility producing CNTs
**Anna-Kaisa Viitanen**, Ana Sofia Fonseca, Joonas Koivisto, Anneli Kangas, Marika Huhtiniemi, Esa Vanhala, Tareq Hussein, Kaarle Hämeri

Identifying, assessing and controlling nanoparticle exposures among U.S. construction workers
**Bruce Lippy**, Gavin West

Comparing workers measured dust exposure with predicted exposures using a NF/FF model, NanoSafer, and the ART exposure assessment tools
**Ismo K. Koponen**, Antti J. Koivisto, Alexander C.Ø. Jensen, Kirsten I Kling, Marcus Levin, Keld A. Jensen

11:30 Studying nanoparticle translocation and behaviour at the human placental barrier using ex vivo and advanced in vitro model systems
**Tina Buerki-Thurnherr**, Stefanie Grafmueller, Pius Manser, Carina Muoth, Wolfram Jochum, Pierre-Andre Diener, Ursula von Mandach, Peter Wick

From comparison tests to recommendations in standardisation for counting nanoparticles by using CPCs
**Carsten Möhlmann**, Christian Monz, Volker Neumann, Dirk Dahmann, Christof Asbach, Heinz Kaminski, Ana Maria Todea

11:45–12:45 Lunch

Room: Fennia II
Chair: Iseult Lynch

12:45 **KEYNOTE 7:** Useful properties of nanomaterials and the development of functional assays for evaluation of nanomaterial exposure and hazard in complex systems
Mark Wiesner, Duke University, USA

13:15 **KEYNOTE 8:** Risk assessment of nanomaterials: Current status and research needs
Andrea Hartwig, Karlsruhe Institute of Technology, Germany

13:45–15:00 **POSTER SESSION 2** (see page 66) + coffee break
Room: Nordia

15:00–16:15 **FREE COMMUNICATION SESSIONS (PARALLELS FC7 + FC8)**

**FC7: ECOTOXICOLOGY AND NEW MEANS FOR NANOMATERIAL TOXICITY IDENTIFICATION**
Room: Fennia II
Chair: Anne Kahru

15:00 Genetic changes and circulating protein levels in Danio rerio exposed to gold nanoparticles (nAu) in aquatic media
**Tarryn Lee Botha**, Mayumi Ishizuka, Shouta Nakayama, Yoshinori Ikenaka, Victor Wepener

15:15 FullereneC60 loading interferes of the growth and emergence rate of the Midge Chironomus riparius
**Greta C. Waissi-Leinonen**, Inna Nybom, Kukka Pakarinen, Elijah J. Petersen, Jarkko Akkanen, Matti T. Leppänen, Jussi V.K. Kukkonen

15:30 NanoMILE at the halfway point: mechanistic insights and progress towards a grouping and classification framework
Eugenia (Eva) Valsami-Jones, **Iseult Lynch**

**FC8: RISK ASSESSMENT AND CONTROL**
Room: Fennia I
Chair: Helene Stockmann-Juvala

Risk assessment of inhalation exposure to engineered nanomaterials
**Antti J. Koivisto**, Kai Savolainen, Hannu Norppa, Harri Alenius, Ismo K. Koponen, Keld A. Jensen, Kaarle J. Hämeri

A multiple perspective framework for the grouping of nanomaterials
Josje H.E. Arts, Mackenzie Hadi, Athena M. Keene, Reinhard Kreiling, Delina Lyon, Monika Maier, Karin Michel, Thomas Petry, Ursula G. Sauer, David Warheit, Karin Wiench, **Robert Landsiedel**

The collective protection against nano-objects
**Elżbieta Jankowska**, Tomasz Jankowski, Celina Vaquero, Jesús López de Ipiña, Ben Hargreaves, Ioan Pepenar, Adrian Ţabrea, Maria Jaen, Íñigo Larraza, Raúl Pina

15:45 **Standardized in vitro high-throughput screening analyses serve efficiently for rapid ranking of nanomaterials toxicity under diverse testing protocols**
**Vesa Hongisto**, Roland Grafström

16:00 **High content imaging reveals cell type-specific nanotoxicity**
**F. Joris**, K. Braeckmans, J. Demeester, S.C. De Smedt, K. Raemdonck

**Level of control self-assessment method for handling of engineered nanomaterials**
**Arto Säämänen**, Tomi Kanerva, Virpi Väänänen

**Penetration of engineered nanoparticles through nitrile rubber gloves**
**Ludwig Vinches,** Mohamed Zemzem, Caroline Peyrot, Stephane Halle, Kevin James Wilkinson, Nathalie Tufenkji

Room: Fennia II

16:15 **PLENARY: INTERACTIVE DISCUSSION**
**"Safety assessment of ENM on regulatory perspective "**
Moderator: Vicki Stone, Heriot-Watt University, UK
Invited commentary: Maila Puolamaa, EC

17:00 End of day

19:00–23:00 Congress Party
Venue: Restaurant Zetor
Address: Mannerheimintie 3-5, Helsinki
Price: EUR 60 /person

# Wednesday, 15 April 2015

**8:30–13:00** **Registration and information at Marina Congress Center**

**Room: Fennia II**
**Chair: Harri Alenius**

**9:00** **KEYNOTE 9:** Use of Alternative Test Strategies, Predictive Toxicological Approaches and Categorization to expedite Decision Analysis of Nanomaterial Safety
Andre Nel, UCLA, USA

**9:30** **KEYNOTE 10:** The IARC Monographs' evaluation of the carcinogenicity of carbon nanotubes
Kurt Straif, IARC, France

**10:00** **Break**

**10:30–12:00** **Parallel satellite workshops (A and B)**

**Room: Fennia II**
**WORKSHOP A: TOXICIOLOGY**
**Chair: Harri Alenius**

- Introduction
- Anna Shvedova: "Risk of Altered Immune Function Associated with Expanding Production and Use of Novel Nanomaterials"
- Requested commentators
- General discussion

**Room: Fennia I**
**WORKSHOP B: RELEASE**
**Chair: Derk Brouwer**

- Introduction
- The relevance of standardized release testing as functional/ behavioral property testing (invited speaker)
- Poster pitches
- General discussion

**Room: Fennia II**
**Chair: Kai Savolainen**

**12:00** **KEYNOTE 11:** The role of safety and trust in promoting global benefits of responsible nanotechnologies
Sirirurg Songsivilai, National Nanotechnology Center, Thailand

**12:30** **EU Greetings – NanoSafety Cluster review**
Georgios Katalagarianakis

**12:50** **Poster awards**

**13:00** **Closing words**
Kai Savolainen, FIOH

# Social programme

***Get-together***
Sunday, 12 April 2015 at 18:00 - 20:00
Venue: Scandic Grand Marina Hotel, Room Commodore (lobby floor)
Address: Katajanokanlaituri 7, Helsinki
Price: Included in delegate fee

***Helsinki City reception***
Monday, 13 April 2015 at 19:00 - 20:30
Venue: Helsinki City Hall
Address: Pohjoisesplanadi 11 - 13, Helsinki
Price: Included in delegate fee

***Congress party***
Tuesday, 14 April 2015 at 19:00 - 23:00
Venue: Restaurant Zetor
Address: Mannerheimintie 3-5, Helsinki
Price: EUR 60 /person

# FURTHER EDUCATION LECTURES

## Lecture 1A: Physico-chemical characterisation of nanoparticles in suspension and embedded in biological matrices

**ERIK H. LARSEN,** Technical University of Denmark, National Food Institute, The Nano-Bioscience group, Denmark

Before initiation of biological and toxicological studies with nanoparticle powders, they need to be suspended in a dispersant. For the sake of reproducibility within and between laboratories, the ultrasound equipment which is often used for breaking agglomerates and dispersing the nanoparticles should ideally be calibrated such that the delivered acoustic power (watts) is known. It is described how this calibration is carried out by the so-called calorimetric method.

Once in suspension, the size distribution of the nanoparticles can be determined by a variety of techniques including dynamic light scattering or multi-angle light scattering and the and number concentration and its size distribution can be determined by transmission electron microscopy or single-particle ICP-MS. Equally important, the time-resolved stability of nanoparticles must be assured by repeated analyses over the duration of the experiment in which the nanoparticles are being used. Only under such circumstances, is it possible to report the size and number concentration, as well as the mass concentration administered in a biological experiment, thereby making possible a sound interpretation of the results or end-points that are measured in the experiment.

Following harvest of a cell-line or sacrifice of an animal, various schemes exist for liberation of the nanoparticles embedded in cells or tissues. Such methods involve gentle degradation of the biological sample by enzymes or by alkaline hydrolysis. The pH value or the ion strength of the extract however, may cause agglomeration of the nanoparticles in the extract, and therefore no longer represent the original state of the nanoparticles in the biological matrix. Stabilisation by surface coating of the NPs or surrounding by a protein corona may however, help stabilizing the NP suspension.

Physico-chemical characterization of NPs in the extract may then be determined in batch mode, e.g. by DLS of the hydrodynamic diameter or the nanoparticle projected area by TEM. Disturbances from matrix constituents however, may render results from such non-specific techniques hard to interpret. The mass concentration of nanoparticles can be determined by molecular or atomic spectrometric detection techniques such as UV absorbance or AAS and ICP-MS. The selective detection techniques (AAS and ICP-MS) often lead to highly accurate and precise results as the influence from sample matrices is minimum. This is an obvious advantage in ADME studies of NPs following dosage in an animal model.

Larger selectivity can be achieved by using coupled techniques such as separation of an NP mixture by field-flow fractionation with detection by DLS/MALS and ICP-MS.

A single technique for characterization is most cases too limited to provide the necessary information to interpret the effects of NPs on biological end-points. This tutorial course will emphasize the need for having many tools in the tool box to provide information not only on mass concentration, size distribution, but also to gain information on surface reactivity and fine structure of NPs.

This course will, in a dialogue style, provide a range of examples from the authors own research. The issue of quality assurance of the produced results from physico-chemical methods of analysis will be highlighted.

## Lecture 1B: Introduction to systems nanotoxicology

**DARIO GRECO,** Unit of Systems Toxicology and Nanosafety Research Centre, Finnish Institute of Occupational Health, Finland

Engineered nanomaterials (ENM) are incorporated in many consumer products and human exposure increases as the development of new ENM proceeds. However, the features that make ENM desirable in various applications have also the potential to alter the biological properties impacting their safety. The novel field of systems nanotoxicology aims at studying the nano-bio interactions at multiple levels by comprehensive molecular profiling of the exposed cells, tissues and organisms. The aim is to model the effect of ENMs taking into account the intrinsic physico-chemical characteristics of the materials in order to help the development of new safe-by-design ENM.

In the occupational context, ENM enter the organism mainly via the airways by inhalation, but still little is known of their early effects on pulmonary allergic diseases. We recently observed that rod-like carbon nanotubes are able to induce allergic asthma in mice when inhaled for 4 hours. Moreover, differently shaped carbon nanotubes evocate diverse transcriptional and functional responses already after a single exposure. In the context of the EU FP7 project NANOSOLUTIONS, we are coordinating the systems biology work package with the task of developing a computational classifier able to predict the safety of ENMs.

## Lecture 2A: Occupational exposure assessment - Measurement, modeling and control banding

**KELD ALSTRUP JENSEN,** The Danish Nanosafety Centre, National Research Centre for the Working Environment, Denmark

Proper exposure assessment of manufactured nanomaterials is becoming a critical issue. Recommendations for new occupational inhalation exposure limits are emerging. Provisional exposure limits or action limits are proposed in different organizations and countries. New exposure metrics is under discussion and includes the use of aerosol particle number concentrations and surface area. At the same time several workplace measurements using several different types of equipment have documented that exposure to nanomaterials do occur. But very few of these measurements quantify the exposure well and uncertainties have arisen on the comparability and reliability of the results obtained from different real-time aerosol measurement devices. In lack of proper measurements, modeling may be used, even in regulatory contexts, to assess worker exposure. However, tools recommended in the European Chemicals Agency guidance document R.14, were not made for nanomaterials, which creates uncertainty about their assessment results. To ensure a safe working environment, exposure (and risk) management may be reached by application of precautionary Control-Banding tools.

In the first part of this lecture, examples of nanomaterial workplace exposure measurement studies will be presented and discussed in regard to observed exposure levels, influence of different sources, and the reliability of different typically applied online measurement devices. In the second part of the lecture, I will discuss and demonstrate the use of modeling tools as recommended in REACH Guidance document R.14 and select Control Banding Tools with focus on the Dutch Stoffenmanager Nano and the Danish NanoSafer.

Please bring your own lab-top for use in an exercise!

## Lecture 2B: Cardiovascular effects of nanomaterials

**FRITZ KROMBACH,** Ludwig-Maximilians-Universität München, Germany

Recent research has demonstrated that the cardiovascular system is an important target for nanomaterial toxicity. Proposed mechanisms responsible for these effects include inflammation, oxidative stress, autonomic dysregulation, and direct interactions of nanomaterials with blood components and cells of the cardiovascular system.

The first part of this tutorial presentation will highlight potential cardiovascular endpoints associated with pulmonary and systemic routes of nanomaterial exposure and give an overview of the mechanisms of toxicity discussed in the current literature.

The second part of the lecture will more closely focus on the interactions of nanomaterials with blood (leukocytes, platelets), endothelial, and tissue cells, the fate and distribution of nanomaterials in the microcirculation and in tissue, and the effects of nanomaterials on microcirculatory function, thrombus formation, and leukocyte recruitment.

## Lecture 3A: Approaches for predictive nanotoxicology: Feasibility and challenges for risk assessment of nanomaterials

**KARIN ASCHBERGER,** JRC-IHCP Systems Toxicology Unit, Italy

Authorities and companies have to safeguard human and environmental health when it comes to the increasing use of nanotechnology and number of nanomaterials produced.

Nanomaterials (NMs) exibit an additional level of complexity compared to bulk materials, as they may be defined depending on their size, forms, manufacturing method and surface modification. A case by case risk assessment of all different forms based on current approaches is unfeasible because of financial and time constraints. In addition several regulatory frameworks encourage or request the reduction or abolition of animal tests.

Integrated assessment approaches use data generated by multiple alternative methods combined with other types of information. Such alternative in vitro and in silico methods and approaches include adverse outcome pathways (AOPs), quantitative structure activity(property) relationships (QSAR/QSPR), physiologically based kinetic (PBK) modelling, chemical grouping and read-across.

AOP is a conceptual framework that provides a structured overview of relevant physiological processes that are sequentially linked and that can be perturbed by stressors at different levels of biological organisation, leading to an adverse outcome. Read-across and QSAR in combination with PBK modelling approaches can be exploited to identify possible descriptors that could predict the fate, behaviour and adverse effects of NMs. This can be supported by in vitro investigations in an AOP context.

Such approaches are already partly applied in regulatory risk assessment, however they are mostly based on chemical' induced effects and still have to be adapted to NMs and their specific properties. The presentation will show current knowledge and challenges in applying these approaches.

## Lecture 3B: Environmental effects of synthetic nanomaterials: nanoecotoxicology

**ANNE KAHRU,** Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Estonia

Nanotechnology has become a field of great significance—society expects nanorevolution to make fast advances in many fields from space exploration to agriculture. One example, for instance, is the growing use of ZnO and TiO2 nanoparticles as UV-blockers in sunscreens and the use of copper and silver nanoparticles in biocidal products. According to the Nanotechnology Consumer Products Inventory (www.nanotechproject.org) by October 2013 there were there were more than 1600 nanotechnological consumer products on the market in October 2013 representing a 24% increase since the last update in 2010.

Breaking down a substance to nano size (< 100 nm) increases its specific surface area up to millions of times, making it more reactive and thus more powerful for different novel applications, but also potentially more poisonous. The increasing use of nanomaterials (whatever the application) will inevitably lead to their release to the environment in various stages of their life cycle.

Notably, similar to the development of ecotoxicology, which has been severely lagging behind toxicology, nanoecotoxicological research lags behind nanotoxicology by at least 10 years (Kahru and Ivask, 2013).

This presentation will give the overview on current scientific knowledge regarding the ecotoxicity of engineered nanomaterials with a focus on aquatic freshwater species of different food-chain level.

Particular emphasis will be drawn on metal-containing nanomaterials e.g., dissolution, bioavailability and (eco)toxicity.

Acknowledgements: support by IUT 23-5, FP7 projects NanoValid and Modern is acknowledged

References: A. Kahru and A. Ivask (2013). Mapping the Dawn of Nanoecotoxicological Research, Accounts of Chemical Research, 46: 823–833.

# KEYNOTE PRESENTATIONS

## Monday

### Keynote I: Opportunities and challenges of carbon-based nanomaterials

**MAURIZIO PRATO,** Università degli Studi di Trieste, Italy

Carbon nanomaterials, including carbon nanotubes and graphene, have attracted a lot of attention, due to their extraordinary physical and chemical properties, their potential applications and their controversial toxicological profile. Our group has been involved in the organic functionalization of various types of nanocarbons, including carbon nanotubes, fullerenes and, more recently, graphene. The organic functionalization offers the great advantage of producing soluble and easy-to-handle CNTs. As a consequence, since biocompatibility of CNTs is improved, many functionalized carbon nanotubes may find useful applications in the field of nanomedicine.

CNT functionalized with bioactive moieties are particularly suited for targeted drug delivery. In fact, not only they exhibit reduced toxicity, but also possess a high propensity to cross cell membranes. Carbon nanotubes and graphene can also act as active substrates for neuronal growth, a field that has given so far very exciting results. Nanotubes are compatible with neurons, but especially they play a very interesting role in interneuron communication. Improved synaptic communication is just one example.

During this talk, we will report the latest advances of the most exciting results obtained in our laboratory in these fast developing fields.

### Keynote 2: Nanomaterials: One viewpoint of a materials scientist

**OLLI IKKALA,** Aalto University, Finland

This talk reviews some of the recent developments related to functional nanomaterials, where the emphasis is in compositions involving nanoscale particles of different sizes, shapes, and compositions. One of the materials that have aroused considerable recent interest deals mechanically strong nanocelluloses, which consist of a few nanometer diameter fibrils of native crystal forms of cellulose, as extracted from plants or wood. Depending on the processing conditions, they can be either rod-like of 50 -300 nm length or longer and entangled. Several forms of surface functionalizations have been developed. Such materials are used in bulk composites, films, lightweight porous aerogels, and scaffolds to grow even stem cells. Therefore, at least some forms are inherently highly biocompatible. More classically, carbon nanotubes, graphene, and fullerenes are widely explored for applications. Especially carbon nanotubes are used as semiconductors in soft electronics and graphene as conductors. Fullerenes are applied in photovoltaics. Also new types of inorganic 2-dimensional materials have been introduced recently. Various metal nanoparticles have applications in optical applications, such as plasmonics. Finally, semiconducting "hair-like" surfaces allow sensing. This talk aims to give a materials scientist´s point of view on the feasibility and perspectives on nanomaterials for the experts of safety.

### Keynote 3: Opportunities and risks of nanomaterials – What can we learn by looking through a magnifying glass?

**BARBARA ROTHEN-RUTISHAUSER,** University of Fribourg, Switzerland

The increased production and use of engineered nanomaterials makes it crucial to understand their interaction with biological systems. Extensive literature is available on the synthesis and characterization of nanomaterials, however, fundamental understanding on the interaction of nanomaterials at the single cell level is still limited but can provide essential information pertaining to the potential reactivity of any nanomaterials.

One has to be aware that the cellular interaction of nanomaterials depends upon their physicochemical properties (i.e. size, shape, and surface charge) and that these properties might change in complex biological environments. In addition, cellular systems are very complex and each cell type can react very differently to the same nanomaterial. To investigate nanomaterial-cell interactions at a fundamental level, it is crucial to work with extremely well-controlled and characterized materials as well as standardized cell culture systems, necessitating the application of various cutting-edge analytical and imaging techniques while keeping in consideration their limitations and pitfalls.

### Keynote 4: Taking stock of the current status of medical surveillance and epidemiologic research for nanomaterial workers

**PAUL A. SCHULTE,** National Institute for Occupational Safety and Health, United States
RALPH ZUMWALDE, National Institute for Occupational Safety and Health, United States
DOUGLAS B. TROUT, National Institute for Occupational Safety and Health, United States
MARY SCHUBAUER-BERIGAN, National Institute for Occupational Safety and Health, United States
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Since 2000, scientific research has shown adverse effects in animals exposed to some engineered nanoparticles and now after roughly 15 years of commercialization, it is useful to again take stock of current medical surveillance practices for nanomaterial workers. In 2009, NIOSH guidance concluded that "currently there is insufficient scientific and medical evidence to recommend specific medical screening of workers". The guidance called for hazard surveillance and continued use of established medical surveillance practices. The current driver for medical surveillance is the growing number of animal studies linking engineered nanoparticle exposure to adverse health effects. While not conclusive about the potential health risks to humans, the results from these studies begin to point to what health effects might be assessed in workers. However, overview reports indicate that non-targeted medical surveillance and screening for sentinel events has been the practice. It is useful to consider what type of evidence would be needed upon which to build specific medical surveillance or screening recommendations. While some of this evidence will be from animal studies, epidemiologic research will be a major contributor. Early cross-sectional studies of workers indicate that some nonspecific biomarkers such as various antioxidant enzymes and cardiovascular markers may potentially be useful. There are no long-term prospective human studies that link biomarkers and occupational exposure to engineered nanoparticles to health effects. There has been a call for a globally harmonized strategy for how to proceed with epidemiologic research. A framework strategy has been developed and will be reviewed.

## Tuesday

### Keynote 5: Collection and characterization of released nanomaterials from nano-enabled products: NANOSOLUTIONS Case Studies

**SOCORRO VÁZQUEZ-CAMPOS,** LEITAT Technological Center, Spain
ALEJANDRO VÍLCHEZ, LEITAT Technological Center, Spain
ELISABET FERNÁNDEZ-ROSAS, LEITAT Technological Center, Spain
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The potential impacts of engineered nanomaterials (ENM) on humans and the environment have generated considerable research interest, since its use and diversity of applications in commercial products have grown extensively over the past decade. However, one of the main concerns that still needs to be addressed is the hazard impact caused by nanomaterials released during the different life cycle stages of nano-enabled products.

NANOSOLUTIONS project main goal is to develop a hazard classifier that will allow predictions of Hazard based on physicochemical properties of the ENM. Several nano-enabled products have been selected to study in the project, more specifically to identify those processes during their life cycle in which the release of NM is more likely to happen. Furthermore, quantification and characterization of these released NM and the evaluation of their hazard profile will be covered. This presentation will show the results obtained for the collection and characterization of released NM from some nanoenabled products

(textiles, inks, ...) in NANOSOLUTIONS case studies. First of all, setups for simulations of identified life cycle stages in which release will be more likely to occur, will be described. Additionally, the physico-chemical properties of these released NM will be presented, including the properties of those ENM that have been incorporated in the final products.

## Keynote 6: Genotoxicity of nanomaterials: challenges and nanospecificity

**HANNU NORPPA,** Nanosafety Research Centre, Finnish Institute of Occupational Health
KIRSI M. SIIVOLA, Nanosafety Research Centre, Finnish Institute of Occupational Health
GERARD VALES, Nanosafety Research Centre, Finnish Institute of Occupational Health
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KAI M. SAVOLAINEN, Nanosafety Research Centre, Finnish Institute of Occupational Health

Genotoxic nanomaterials can roughly be divided in poorly soluble (inert) and partly soluble nanoparticles. The genotoxicity of poorly soluble particles is usually considered to be indirect, mediated by reactive oxygen species, depending on such variables as particle size, shape (aspect ratio), rigidity (fibres), surface activity and charge, dispersibility, cellular uptake, and biopersistence. In vitro, the genotoxic effect of poorly soluble nanoparticles in cells that have taken up nanoparticles is often relatively low, but may be persistent or continuous. If this is also true in vivo, a low-level induction of genotoxic effects of biopersistent particles could continue for a long time, which may have significance in carcinogenesis. Secondary genotoxicity associated with prolonged inflammation is another possible source of longer-term (and systemic or "non-targeted") genotoxic effects in vivo. The genotoxicity of partly soluble nanoparticles (expected to be less biopersistent than poorly soluble ones) also depends on particle characteristics but may especially be determined by the soluble form and the Trojan horse effect: size-dependent cellular uptake as particles and subsequent dissolution inside the cell to a genotoxic form. The present study summarizes experience gained from studies on the genotoxicity assessment of nanomaterials, with examples from of, e.g., the poorly soluble TiO2 and carbon nanotubes and the partly soluble ZnO and CuO. One of the key questions in nanogenotoxicology is whether nanosized materials have specific genotoxic effects or higher genotoxicity in comparison with similar material of larger size. (Supported by the Finnish Work Environment Fund)

## Keynote 7: Useful properties of nanomaterials and the development of functional assays for evaluation of nanomaterial exposure and hazard in complex systems

**MARK R. WIESNER,** Duke University, United States

The observation that some materials exhibit properties at the nanoscale that may differ from the properties observed for bulk materials is at the very heart of many applications of nanomaterials. The properties of nanomaterials that inspire their use in a rapidly growing number and variety of products, therefore provide a starting point for categorizing engineered nanomaterials (ENMs) for the purpose of identifying ENMs with greater or lesser potential for risk to human health and the environment. The properties that motivate the use of nanomaterials will likely determine exposure paths and production amounts. For example, ENMs used for their photocatalytic properties are more likely to be applied as surface coatings while ENMs having desirable strength properties will more likely be embedded within composites.

The practical implementation of a categorization scheme for nano EHS, building on distinctions between the useful properties of nanomaterials requires that these properties be quantifiable and that relationships between useful properties and EHS impacts (if they exist) be identified. Functional assays are procedures for quantifying parameters that describe a specific process (or function) occurring within a given (often complex) system, typically providing information on the rates at which these processes occur or final outcomes (e.g., air/water, living/dead). This presentation provides the motivation for functional assays and discusses two examples of functional assays targeted on one hand for evaluating the photocatalytic of nanomaterials, and on the other the environmental transport characteristics.

### Keynote 8: Risk assessment of nanomaterials: Current status and research needs

**ANDREA HARTWIG,** Karlsruhe Institute of Technology (KIT), Germany

Nanomaterials are widely distributed in the environment and at workplaces. Nevertheless, the issue of risk assessment appears to be far behind the growing field of applications and is yet important to support the manifold potentials of nanotechnology. Even though there have been increasing numbers of publications related to the potential toxicity of nanomaterials, their use for quantitative risk assessment is often limited due for example to unrealistic exposure conditions, both in vitro and in vivo. One principal question to be answered is whether or not there are modes of action and/or target organs unique for nanomaterials as compared to particles in the microscale range, for example when comparing granular biopersistent particles. With respect to metal-based particles, differences in the uptake as well as extra- and intracellular release of metal ions appear to be relevant. Since all nanomaterials exert adverse effects to some extent, a ranking of nanomaterials from low to high toxicity is required, based on dose-response relationships with respect to exposure and biological effects and taking into account toxicokinetic and toxicodynamic interactions. Since it is neither feasable nor desirable to perform long-term in vivo studies with all nanomaterials, suitable toxicogical endpoints and criteria need to be defined to perform a classification of nanomaterials as a basis for the establishment of environmental and occupational exposure limits.

## Wednesday

### Keynote 9: Use of alternative test strategies, predictive toxicological approaches and categorization to expedite decision analysis of nanomaterial safety

**ANDRÉ NEL,** UCLA, USA

With the advent of the next chapter of nanotechnology ("Nano 2") in the USA, there is an expectation to get more nanomaterials and nano-enabled products to the marketplace, requiring expedited risk assessment and ability to make decisions that can assist assist Nano EHS governance. This necessitates an integrated approach, in which the ability to decide should be premised on expedited testing, grouping and a predictive approaches towards nanomaterial safety that addresses material categories instead of relying on individual materials only. Key to building the incremental knowledge domain is the consideration of how engineered nanomaterial (ENM) physicochemical properties relate to events at the nano/bio interface and how to use this structural and mechanistic information to perform more rapid screening, hazard ranking, establishment of structure-activity relationships, grouping and decision analysis. I will delineate the development of predictive toxicological approaches to assess hazard and risk assessment of ENMs by tiered testing approaches that pave the way to the use of alternative test strategies (ATS) for large category screening. I will provide illustrative examples of the use of adverse outcome pathways to provide high content and high throughput screening of categories of ENMs to illustrate this approach and demonstrate how the data can be used for preliminary rounds of material categorization and assessment of quantitative structure-activity relationships. I will demonstrate how ATS can assist the development of qualitative and quantitative risk assessment approaches, including integrated decision analysis to propose a comprehensive approach to nanomaterial safety assessment, safer design and regulatory decision-making.

### Keynote 10: The IARC Monographs evaluation of the carcinogenicity of carbon nanotubes (Vol. 111, October 2014)

**KURT STRAIF,** IARC, France

In October 2014, IARC convened a Monographs meeting to assess the carcinogenicity of carbon nanotubes (CNTs).
CNTs may consist of either a single graphene cylinder (SWCNTs) with an outer diameter of 1–3 nm, or of multiple graphene cylinders arranged in concentric layers (MWCNTs) with diameters of 10–200 nm. CNTs are typically few micrometres in length, ranging from a few hundreds of nanometers to several tens of micrometers.

Regarding carcinogenicity in experimental animals, the WG concluded that there was sufficient evidence for MWCNT-7, limited evidence for the two other types of MWCNTs with dimensions similar to MWCNT-7, and inadequate evidence for SWCNTs.

Mechanistic data in rodents demonstrated translocation of MWCNTs to the pleura. Inhalation of some MWCNTs or SWCNTs induced acute or persistent pulmonary inflammation, granuloma formation, fibrosis, and bronchiolar or bronchioloalveolar hyperplasia in rodents. Studies in rodents and in cultured human lung or mesothelial cells showed that MWCNTs and/or SWCNTs induce genetic lesions. SWCNTs and MWCNTs also perturb the cellular mitotic apparatus, including microtubules and centrosomes, in human lung epithelial cells.

The WG acknowledged that the above mechanisms are relevant to humans. However, a majority did not consider the mechanistic evidence for carcinogenicity to be strong for any specific CNT. Furthermore, the lack of coherent evidence across the various distinct CNTs precluded generalisation to other types of CNTs. Thus, MWCNT-7 was classified as possibly carcinogenic to humans (Group 2B); and SWCNTs and MWCNTs excluding MWCNT-7 were categorized as not classifiable as to their carcinogenicity to humans (Group 3).

## Keynote 11: The role of safety and trust in promoting global benefits of responsible nanotechnologies

**SIRIRURG SONGSIVILAI,** National Nanotechnology Center, Thailand

Public acceptance of emerging technologies is crucial for the further development and integration of such technology in the society. Nanotechnology, over the past decades, has been applied in the development of various products and services. Recent survey of the global risk landscape still puts the unforeseen consequence of nanotechnology as one of the concerns. In order to facilitate sustainable utilization of nanotechnology, public understanding and trust especially on the safety, benefits and limitations of nanotechnology should therefore be integrated in all stage of the development and product release. This includes knowledge management, establishment of sound and appropriate regulations, and public engagement. The community should encourage the participation of stakeholders and society in the early and every stages, promote transparency and disclosure of information and continuous communications.

# Oral free communications | FC1

## Fibre-induced gene and microRNA expression changes in human lung cells: a comparative study on MWCNTs, asbestos and glass wool

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The high aspect ratio (HAR) of certain engineered nanomaterials (ENM), e.g. multi-walled carbon nanotubes (MWCNTs), is of concern due to the possible induction of cytopathological effects similar to those of asbestos fibers. Most existing data have studied the consequences of exposures coupled with extensive toxicity. Omics technologies are useful for hypothesis generation related to toxicity mechanisms associated with diverse exposure levels. Moreover, the abundance of data points in omics data potentially permits for read-across among different agents based on their mechanism of action. Considering the above, influences of MWCNTs, asbestos and glass wool were analyzed in the human lung cell line BEAS-2B. The experiments addressed whether asbestos and glass wool could serve as positive and negative controls for evaluating harmful fiber-like effects of MWCNTs. A multitude of commonly used cytotoxicity assays initially indicated that doses between 5 and 350 µg/cm2 induced significant toxicity over treatment periods 4-48 h. A further analysis of mitochondrial membrane potential changes variably indicated influences at even lower levels (0.25-2 µg/cm2) and these doses were further explored by omics technologies. Preliminary transcript and miRNA analyses demonstrated largely distinct toxicity mechanisms between the different agents, even if similarities in time-dependent response-patterns were seen. The miRNA data supported the transcriptomics by pinpointing relevant processes. The current study provides a systematic assessment of the multiple influences of fiber-like materials. Deepened bioinformatics analyses of the multiple gene and miRNA expression changes noted in the current data set have potential to further elucidate the pathogenicity of HAR ENMs like MWCNT.

## Suitability of in vitro tests for the assessment of the toxicity of nanoparticles with surface plasmon resonance (SPR) properties

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Engineered nanoparticles (NPs) with Plasmon resonance (SPR) properties are popular in consumer- and medical-based industries due to their unique surface characteristics. Identifying their toxicity is critical given the increased exposure to these NPs. The toxicity is often determined using conventional colorimetric and optical high-throughput systems that rely on absorbance, luminescence or fluorescence signals, which may be prone to interference by the NPs having SPR properties producing erroneous results.

When the toxicity and genotoxicity of particles with SPR properties were assessed using three cell viability systems and three fluorescent dyes, often used in high-throughput systems, results have indicated that both AuNPs and AgNPs, have interfered with all the toxicity tests implemented and also quenched the fluorescence of the dyes, 2', 7'-dichlorofluorescein (DCF) and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzamidazolocarbocyanin iodide (JC-1). On the other hand, these nanoparticles produced no mutagenicity with the Ames test due to their lack of ability to enter the Salmonella typhimurium bacterial cells, but have interfered with DNA during lyses in the Comet assay. When these nanoparticles were also investigated for their interaction with RNA during isolation, quantification and analysis during gene induction and implementation of the microarray techniques, they showed interaction with RNA during extraction and purification processes for the gene arrays.

Toxicity results should therefore be interpreted with caution when using conventional systems. Moreover, the isolation, quantification, purity and integrity are critical points of analyses for RNA-based techniques and therefore, the observed quenching effect would result in quantification errors and in obscured integrity validation for gene expression studies.

## Can the comet assay be used reliably to detect nanoparticle-induced genotoxicity?

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The comet assay is a sensitive genotoxicity test for the detection of DNA strand breaks as well as oxidatively damaged DNA at the level of single cells. Today the assay is commonly used in the nano-genotoxicology field. The aim of this study was to investigate possible interactions between nanoparticles (NPs) and the assay, and furthermore to compare the outcome in studies in which both the comet assay and the micronucleus (MN) assay have been used. Concerns for NP-comet interactions have arisen from the observation of NPs in the "comet head", which implies that NPs may be present during performance of the assay. Following exposure of Beas-2B cells to CuO NPs, we found induction of DNA breaks when the NPs were added only during the assay performance, indicating that additional damage formed during the assay is possible for some NPs. However, for most NPs, an interaction that substantially can impact the comet assay results is unlikely. Furthermore, exposure of the comet slides to light led to an increase in DNA breaks in cells treated with anatase TiO2 NPs, suggesting that care must be taken when working with NPs with high photocatalytic activity. Finally, a good consistency between the comet assay and MN assay results was found in published studies in general (69%) and it was even higher when excluding the studies on TiO2 NPs (81%). The strong concordance between the assays applied to a range of different NPs implies that both can be trusted in the assessment of genotoxicity of NPs.

## Iron Oxide Nanoparticles: Effects on cellular uptake, cytotoxicity and differentiation of rat bone marrow derived mesenchymal stem cells

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Stem cells therapy has obtained prior attention in the field of regenerative medicine due to its unique capability to self-renew and differentiate into cells of specific lineages. Nanoparticles due to its superior properties and functionalities are widely used in biomedical applications termed as nanomedicine. The interest on use of nanoparticles such as iron oxide nanoparticles in stem cell therapies has increased rapidly due to its remarkable applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, drug delivery, etc. In order to use the iron oxide nanoparticles for these purposes, we firstly need to determine its impact on the functions of stem cells, especially those related to differentiation. This study aims to monitor the effects of two different kinds of iron oxide nanoparticles, with similar size (about 150 nm in water measured by DLS) but different surface coating, on rat bone marrow derived mesenchymal stem cells (RBMSCs). The pristine particles and citric acid modified particles have zeta potential of 27.93 and -44.43 mV respectively measured by DLS. The uptake and intracellular location of both nanoparticles were confirmed by TEM and quantified by ICP-MS. Citric acid modified particles showed stronger cytotoxicity than pristine particles, but the extent is not high. The pristine iron oxide nanoparticles can induce osteogenic differentiation of RBMSCs, revealed by calcium accretion after 14 days. In contrast, the citric acid modified nanoparticles did not show significant impact on the differentiation of RBMSCs.

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### Enzymatic 'stripping' and degradation of PEGylated single-walled carbon nanotubes by neutrophils elastase and myeloperoxidase

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Poly(ethylene glycol) (PEG) modified single-walled carbon nanotubes (SWCNTs) are being considered for use as vectors for drug delivery in nanomedicine. However, it is necessary to understand whether such vectors persist in the body, with potential long-term effects, or can be biodegraded. Therefore, in the current study, SWCNTs coated (cPEG) or, functionalized (fPEG) with PEG chains of different molecular weight (MW) were assessed for their propensity to undergo biodegradation under in vitro conditions using human myeloperoxidase (MPO) and ex vivo using freshly isolated primary human neutrophils. Under in vitro conditions, the PEGylated SWCNTs (PEG-SWCNTs) were found to biodegraded in a time-dependent manner in the presence of MPO supplemented with NaCl and H2O2. However, type of PEG attachment (fPEG > cPEG) on the surface of the SWCNTs and MW of fPEG (2kDa > 5kDa > 10kDa) were found to affect the process of biodegradation as observed using Raman spectroscopy, UV/Vis-NIR spectroscopy and transmission electron microscopy. Under ex vivo conditions, however, all SWCNTs were found to be degraded by the Formyl-Methionyl-Leucyl-Phenylalanine (fMLP) and cytochalasin B agonists stimulated human neutrophils. Neutrophils are known to release significant amount of neutrophil elastase (NE) and other proteases upon activation. Thermogravimetric analysis showed a time-dependent removal of PEG chains from the surface of 5kDa fPEG-SWCNTs treated with recombinant human NE, indicating that this enzyme may cause defunctionalization of PEG-SWCNTs. We therefore speculate that the process of PEG-SWCNTs biodegradation involves the combined action of MPO and neutrophil proteases such as NE.

## Oral free communications | FC2

### Comparison between soft (liposomes) and hard (carbon nanotubes and graphene oxide) functionalised nanomaterials as inducers of in vivo neuroinflammation

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Various nanomaterials have been investigated as platforms for drug delivery to brain regions. One issue regarding the use of nanomaterials in the CNS is the induction of inflammatory responses to the brain parenchyma. This is even more important in view of potential applications of those nanocarriers for the therapy of brain diseases for which neuroinflammation is already a hallmark. To this end, a variety of nanosystems were injected into the striatum of mice brains and the inflammatory response was evaluated at different time points. The data revealed that different nanosystems elicited different patterns of inflammation in the injected area. In particular, lipid-based nanomaterials increased inflammatory marker expression levels, peaking at day 2 and persisting till day 7 after injection, notably for cationic liposomes compared to anionic ones. Administration of carbon nanotubes (oxidised or aminated) or graphene oxide elicited an up-regulation of pro-inflammatory markers only at day 2, reaching a level comparable to the negative control after one week. We monitored the diffusion of the inflammatory response around the site of injection. We found that while administration of carbon nanomaterials revealed a similar trend to the negative control at all time-points, a pro-inflammatory response was instead observed for cationic liposomes. The present findings point out a sustained inflammatory response after intracerebral administration of liposomes regardless of their surface charge characteristics, while carbon-nanomaterials, especially graphene oxide, seem well-tolerated by the brain parenchyma, eliciting insignificant production of inflammatory mediators. These observations suggest that carbon

nanomaterials can be considered as non-inflammogenic materials in applications that may involve injection or implantation directly into the brain.

## Comparison of in vitro and in vivo formed protein coronas: Implication for targeting and cellular internalization

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The adsorption of proteins and their layering onto nanoparticle surfaces has been referred to as 'protein corona'. This dynamic process of protein adsorption is currently being extensively evaluated by incubation of nanoparticles (NPs) with plasma proteins. However, the extrapolation of in vitro formed protein corona to predict the fate of NPs in vivo remains largely untested, therefore comparison of in vitro and in vivo formed protein corona formation on the same NP is of great importance. The aim of this study was to evaluate in vivo protein corona formation of intravenously administered liposomes. Liposome-forming in vivo protein corona was characterized, by recovering the liposomes from the blood circulation of CD-1 mice 10 min post-injection. In comparison, in vitro protein corona was allowed to form by the incubation of the same liposomes in CD-1 mouse plasma. Protein coronas formed in vivo and in vitro were compared in terms of morphology, composition and cellular internalization. Overall, even though the total amount of protein content on the corona of circulating liposomes in vivo correlated with that observed from in vitro incubations, protein species adsorbed in vivo were considerably more. There is no previously reported study in the literature describing the comparison between the protein coronas forming in vitro and in vivo on the same type of nanoparticle.

## Development of nanomaterial libraries for nanosafety studies: Polyvinylpyrrolidone (PVP) Capped Metal Oxide Nanoparticles

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The increased use of manufactured nanoparticles (MNPs) results in increased environmental release, hence concerns that exposure of humans and other organisms is inevitable. The potential environmental impact of MNPs is poorly understood, and the need to better understand MNP fate and transformations is particularly urgent. The aim of this work is to develop and fully characterise a library of comparable nanoparticles with a range of core chemistries, but the same capping, in order to test the hypothesis that the core chemistry is a primary factor in controlling toxicity.

PVP capped ceria nanoparticles were prepared according to Merrifield et al. (2013). The synthesis protocol was successfully modified to produce PVP capped zinc oxide, PVP capped copper oxide, PVP capped zinc doped ceria and PVP capped copper doped ceria MNPs. Our working hypothesis is that the mechanism of MNP formation is the same in all cases. This suggests that the protocol is very robust and has the potential to generate a wide range of comparable MNPs. A range of MNP sizes (c. 5, 7 and 20 nm) was obtained by varying the type and amount of PVP used.

Characterisation was carried out by means of DLS, Zeta potential, UV/VIS, electron microscopy, FT-IR, XRD and XPS so as to confirm the success of the synthesis, its modification and the ceria doping. Particular emphasis was given on the valency state determination of the metal forming the core.

Results to date suggest that the tested protocol can be successfully used to create stable PVP capped metal oxide and doped metal oxide nanoparticles of reproducible sizes and that the hypothesis of a common mechanism holds true. The next stage of the research involves testing the toxicity of the entire library in in vitro experiments.

## The role of surface functionalization in the genotoxicity of different nanomaterials in vitro

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A fundamental goal in nanotoxicology is to understand which nanomaterial characteristics could determine their hazard to human health. This question also concerns the assessment of genotoxicity, an endpoint which is used in identifying carcinogens in regulatory toxicology, widely using in vitro approaches. A key feature in the interaction of nanomaterials with biological structures is surface functionalization. We have studied the role of various surface coatings in the induction of genotoxic effects in vitro. We analyzed the genotoxic potential of several types of nanoparticles (including copper oxide, titanium dioxide and silver) with funtionalizations representing different surface charges: -COOH (negative charge), -NH2 (positive charge) and –PEG (polyethylene glycol; neutral charge) in human bronchial epithelial BEAS 2B cells. The comet assay, after a 24-h treatment, was used to detect DNA damage and the micronucleus assay, after a 48-h treatment, to assess chromosome damage. The dispersions of the nanoparticles in culture medium were characterized by dynamic light scatter. Our preliminary results indicate that the genotoxicity of some nanoparticles is dependent on the surface charge. (Funded by EU FP-7 NANOSOLUTIONS, Grant Agreement No. 309329)

## Who will produce the safe nanomaterials? University teaching content in nanotechnology studies does not match job skill demands in the nanotechnology industry

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The introduction of nano-enabled products into a multitude of markets has led to a strong demand for a highly qualified workforce in industries producing or using nanomaterials. Many universities have responded to this development by setting up curricula on nanotechnology or other nanosciences at the level of bachelor, master and PhD studies. The ongoing FP7 supporting action NanoEIS investigates contents of existing study offers, practices for establishing links between universities and industries with respect to teaching, and the job skills that are in demand in the nanotechnology industry.

The results show that the match between curricula contents and job skill demands is poor. University studies mostly put a strong emphasis on traditional, research-driven subjects like characterization/metrology, nanoelectronics and nanostructures/composites. The nanotechnology industry, on the other hand, identifies health/safety issues as the most important area where recruitment is expected both now and in five years. Health and safety appear to be low priorities in university training, and regulation/standardization as well as environment/disposal/recycling get even less attention, despite being rated as highly important job skills for recruitment by industry.

We suggest that strong efforts need to be taken to improve links between universities and industries with respect to training. Direct involvement of industry in teaching is identified as the most effective means to achieve this, which is in agreement with suggestions from students on how to improve their studies.

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# Oral free communications | FC3

## Pulmonary effects and biokinetics of nanoparticles: Interim results of the long-term inhalation study with $CeO_2$ and $BaSO_4$

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Little is known about long-term effects of airborne, poorly soluble nanoparticles (PSP); this question is currently addressed by a long-term inhalation study according to OECD 453. Nanomaterials were selected to represent a range of different biokinetic and biodynamic properties. Results of 1 and 4 week inhalation studies (OECD 412) with CeO2 (NM212) and BaSO4 (NM-220) were used to design the long-term study; interim results of this study are presented here.
Rats inhaled aerosol concentrations of 50 mg/m3 BaSO4 or 0.5, 5, and 25 mg/m$^3$ CeO2 by whole-body exposure for 6 h/day on 5 consecutive days for 1 or 4 weeks with a post-exposure period up to 129 days.

The Ba and Ce burdens of the lungs, lung-associated lymph nodes and extrapulmonary organs were analysed by ICP-MS. Ba Lung burdens after 1 and 4 weeks of exposure were 1.0 and 0.84 mg/lung, respectively, and decreased during post-exposure with a half-time of about a week. The rapid lung clearance was confirmed by IT-instillation of neutron-activated particles. Inhaled CeO2 was cleared from the lung with a half-time of 40 days; at aerosol concentrations higher than 0.5 mg/m$^3$, this clearance was impaired resulting in a half-time above 200 days (25 mg/m$^3$).

Animals were examined by bronchoalveolar lavage (BALF) and histopathology. No morphological changes or significant increases in BALF were detected after short-term exposure to BaSO4. After 13 weeks, a slight increase of neutrophils was observed. After 5 days, Ceria (>0.5 mg/m$^3$) induced an early inflammatory reaction by increases of neutrophils in the lung which decreased with time, with sustained exposure, and also after the exposure was terminated (during the post-exposure period). Concomitantly more mononuclear cells, especially macrophages, were visible in histopathology. Further progression to granulomatous inflammation was observed 4 weeks post-exposure.

## Taquann dispersion method and Direct Injection Whole Body Inhalation system.

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There is a group of multi-wall carbon nanotubes (MWCNT) that contains fibers of 10 to 20 micrometers long, and we reported that one of them induces mesothelioma dose-dependently when intraperitoneally injected to p53 heterozygous mice. We also suggested that non-granulomatous chronic inflammatory microlesions containing well-dispersed fibers are related to mesotheliomagenesis.

However, such MWCNT is an exceptional case that the knowledge of asbestos can be applied. For new nanomaterials in general, there is no pre-existing knowledge about their toxicity. In such case, whole body inhalation (WBI) toxicity study is the first choice to assess the toxicity considering the most important route of exposure to humans. To facilitate the WBI, we developed “Taquann” dispersion method and the Direct Injection system. Mitsui MWNT-7 used as a test sample. It was dis-

persed in tert-butyl alcohol, filtered to remove aggregates/agglomerates, snap-frozen, and vacuumed. Aliquots were injected to the chamber system periodically by compressed air. The length distribution of the MWCNT recovered from the lungs of the exposed mice was similar to the original sample and aerosol in the chamber. Dispersed fibers reached peripheral alveolar spaces. Some fibers were found, on the parietal pleura, phagocytized by macrophages accompanying lymphoid cells with a covering of reactive mesothelial cells. They were similar to the microlesions found in intraperitoneal studies.

Taquann WBI system is relatively inexpensive, easy and safe to operate, and requires small amount of sample. It would be ideal to run WBI studies on new low-product-volume nanomaterials. (Supported by Grants from MHLW, Japan.)

## Integration of micro RNA and transcriptomic sequencing profiles underlying the response of rat lung to inhaled silver nanoparticles

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Engineered nanomaterials are an emerging category of substances that are finding increasing applications in diverse areas such as electronics, pharmaceuticals and consumer products. There are great concerns over the safety of such materials and so the mechanisms through which they interact and alter cellular and tissue function, require detailed characterisation. In this study, we sought to characterise mechanisms underlying the adverse effects of silver nanoparticle (AgNP) exposure in the rat lung. Sprague Dawley male rats were exposed over 4 days to an estimated inhaled accumulated dose of 40µg/rat spark generated AgNPs (20 nm). Next generation sequencing analysis using the Illumina HiSeqTM200 platform was used to assess global microRNA (miRNA) and mRNA expression in lung tissue. RNAseq analysis revealed distinct patterns of gene expression at 24hrs consistent with an activated immune response, including up-regulation of chemokines and inflammatory gene expression. This expression pattern was diminished 7 days post exposure. miRNA sequencing analysis also demonstrated a pattern of expression with more changes at 24hrs as compared 7 days post AgNP exposure. Initial analysis integrating the potential for regulation of mRNA expression by changes in miRNAs has been performed and upon completion may reveal distinct regulatory mechanisms through which AgNP exposure initiates and controls inflammatory process in the rat lung. This information contributes to our understanding of the potential health risks associated with AgNP inhalation exposure and may provide mechanistic insights into the potential risk to humans.

## Towards the prediction of nanoparticle-induced inhalation toxicity: Evaluation of an in vitro macrophage assay

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To asses the safety of the increasing number of nanomaterials (NM) and modifications thereof, grouping, ranking and integrated biological testing is highly desirable. However, current in vitro models revealed little correlation with rat inhalation studies. In previous projects (NanoCare, NanoGEM) promising correlation was found between studies using primary alveolar macrophages (AM) and/or the NR8383 cell line and short-term inhalation studies (STIS) or intratracheal instillation studies (ITI). This work, therefore, was conducted to continue on this issue.

Measuring endpoints of the so-called vector model (cytotoxicity, macrophage activation, TNF release, oxidative burst), and converting these into a sum index, indicates inasmuch NMs interfere with particle clearance and basic immune function of the lung. A total of 28 previously characterized NM (pure and mixed oxides from Al, Ti and/or Zr; various CeO2; unmodified and surface-modified amorphous SiO2; coated ZrO2; graphite nanoplatelets; different organic and Fe2O3 pigments; Ag; ZnO) were tested against micron-sized quartz DQ12 and corundum. Results were compared to the outcome of 18 ITI and 18 STIS. The

ranking of the NM in vitro ranged from ion-shedding silver or ZnO NM, to Al-doped CeO2, over SiO2 and ZrO2 modifications, to SrCo3 and BaSO4 as being least active. While the in vitro ranking in principle matched the in vivo results, a more detailed comparison of in vitro toxicity and STIS data also had to consider particle surface size. Thus, when STIS and vector model data were subdivided into 4 hazard categories, the majority of in vitro tests matched the correct or a neighbouring category.

Overall, the in vitro approach with NR8383 cells appears promising and well suited to compare the hazard potentials of inhaled nanomaterials.

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## Inflammatory response from 52 volunteers after 75 min exposure to laser printer emissions

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**Introduction**: In recent years, adverse effects of laser printer emissions on human health have been discussed, especially in Germany. As several studies found high ultrafine particle (UFP) emissions from some printer models, we exposed 52 volunteers to low (LE) and high (HE) UFP concentrations, generated by laser printers.

**Methods**: Overall, 23 healthy control subjects, 14 patients with mild asthma and 15 persons with self-reported symptoms (SRS) participated. Each participant was exposed over 75 min to low (≈3000/$cm^3$, near background) and high (≈100,000/$cm^3$) UFP emissions in a randomised, cross-over, single-blinded manner. Before and after the exposures serum samples and nasal secretions were collected and analysed for inflammatory markers (IL-1beta, IL-5, IL-6, IL-8, GM-CSF, IFN-gamma, TNF-alpha) by Bio-Plex Pro™ Cytokine Assays and for eosinophilic cationic protein (ECP) by ImmunoCAP.

**Results**: The serum concentration of ECP was increased after both exposures, but not significantly stronger after HE. Cytokine levels in serum showed no significant and consistent pattern of changes. In nasal secretions there was an increase of IL-6 concentrations after both exposures, with a tendency towards greater changes after HE exposure. Overall, the observed biochemical changes did not provide a coherent pattern regarding the groups of individuals with SRS, with asthma, or subjects with bronchial hyperreactivity.

**Conclusion**: Our results do not support the hypothesis that exposure to high levels of laser printer emissions, in terms of ultrafine particle concentrations, stimulates a distinct disease process that correlates with symptoms typically reported by individuals with SRS.

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# Oral free communications | FC4

## Detailed size and chemical characterization of ultrafine particles in workplaces and comparison to toxicity studies with engineered nanoparticles

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In toxicological studies on nanomaterials, model organisms are typically exposed to single nanoparticle types. However it is unlikely that future workers will be exposed to such a uniform mix of particles in any realistic scenario. Further, current workers are already exposed to a certain mixture of ultrafine or inadvertently created (as opposed to engineered) nanoparticles. These particles present a complex background of exposure to which the new potential risk of engineered nanoparticles will be added. Traditionally these background particles have been grouped into fairly gross categories by size. If chemical makeup was considered at all it has only been analyzed as the proportion of each size grouping. It is not possible to directly compare the current levels of ultrafine particle exposure to the new risk information being developed through the toxicological study of engineered nanoparticles, because the detailed size distribution information is not available for specific particles on the basis of chemical composition. This study collected ultrafine and fine particles from workplaces and produced detailed size distributions by chemical constituents. Transmission electron microscopy (TEM) combined with elemental dispersive spectrometry (EDS) combined with automated image analysis has enabled the detailed assessment of particle sizes and chemical makeup. These distributions are directly compared to current toxicological studies in rodents and other existing studies involving study of ambient concentrations of ultrafine particles. Future risk assessment will depend on detailed characterization of the present occupational background exposure to nanoparticles as well as the development of more realistic exposure scenarios for toxicological studies.

## Tiered approach measurement strategies for inhalation exposure to Nanoobjects and their agglomerates and aggregates: Testing the sensitivity of decision criteria

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Currently, a number of tiered-approach strategies to measure and evaluate exposure to nanoobject and their agglomerates and aggregates are proposed. In these approaches more detailed information is collected in each tier. In the basic assessment, workplace measurement results are evaluated to decide whether there is a need for an in-depth assessment. Evaluation criteria or decision rules are different for the various strategies.

In a desk study, the sensitivity and robustness of a number of decision rules were tested, with special focus on the evaluation of scenarios with measurement results from direct reading devices, where both (particle number) concentration during activity and non-activity (background) were collected. Existing studies data sets were grouped by device- exposure scenario combinations and analyzed according to an appropriate method (ARIMA) to analyze time series. The results were used to set parameters values for the ARIMA model to generate new, simulated, data. In total 27 simulation scenario's were considered and each scenario was run 1000 times. In each replication eight decision rules were tested, including the Dutch NanoReference Value (NRV). Results indicate that the scenario determines which decision rule is most sensitive, so instead of an 'one-size-fits –all' approach, scenario-specific decision rules are needed. Application of decision rules to an extended set of real work place data (n=69) showed that the decision rule with the highest sensitivity still results in about 30% false negative decisions. It is recommended to apply the ARIMA method to evaluate time series and include other information in the evaluation.

## Framework to forecast exposure of the next generation of nanomaterials

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The EU-FP7 project FutureNanoNeeds (FNN) aims to develop a systematic framework to assess safety of next generation of nanomaterials (NM) being developed for industrial applications. This includes the development of a methodology to forecast environmental releases, emissions and (human) exposure of next-generation NM within relevant compartments and in different life cycle stages (LC). Significant input is also received from the nano-enabled novel value chains (VCs) elaborated on within the FNN project.

Based on existing life cycle inventory methodologies, a framework is presented to assess material flows along different life cycle stages. A two-tiered iterative approach is proposed. First, a Tier 1 assessment considers material flows and emissions into compartments along the entire life cycle – intended to identify 'focal points' of concern. Second, the Tier 2, is a more in-depth assessment of the focal points and focuses on specific processes in a NM life cycle. Using an expert elicitation process, Bayesian networks are developed to forecast the NM release, their characteristics, emission and exposure, including possible transformations of the nanomaterials during the whole life cycle. A major advantage of using Bayesian networks is their ability to deliver stochastic information with their prediction and their usefulness in dealing with data and knowledge gaps.

Both the Tier 1 and Tier 2 assessment are illustrated using the case study identified in the value chain (VC) development. Specific criteria to define the focal points which link Tier 1 to the Tier 2 will be presented. Mayor issues that were encountered during the framework development and case studies will be highlighted.

## Mapping occupational exposure to MNMs in construction

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The incorporation of manufactured nanomaterials (MNMs) in construction has leads to concerns about potential occupational exposure to these materials. In this sector, exposure to different kinds of dust is extremely common and it is likely that the exposure to mixed types of dust result in much higher exposure levels than the exposure to MNMs which are normally added in very low concentrations (typically below 1,5 %).

This work presents the results of the measurements of occupational exposure performed during project SCAFFOLD (GA 280535), in scenarios covering 5 selected nano-objects and 6 applications representative of the sector: depollutant mortar (nano-TiO2), self-compacting concrete (nano-SiO2), self-cleaning coating (nano-TiO2), coatings (CNF), fire retardant panels (nano-clays), and insulations (nano-cellulose). The measurements performed cover different steps of the life cycle of these applications.

The main findings can be summarized as follows: 1) Considering the release of particles, no striking differences have been observed for tasks performed with control materials and with materials filled with MNMs; 2) Occupational exposure measured (mass concentration) to nano-TiO2 and CNF was below proposed limits by NIOSH and SCAFFOLD; 3) SEM analysis of samples at the PBZ during machining tasks showed no differences between materials control and filled, and no free nano-objects were identified.

These results are useful for mapping the occupational exposure to MNMs in construction and contribute to clarify the discus-

sion about whether the incorporation of nano-objects to the construction industry may originate an increase on the risks for workers in this sector.

### Process-generated nanoparticles, ignored and uncomfortable sources of workplace exposure to nanoparticles

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Workplace risk assessment of manufactured nanomaterials (MNMs) is generally complicated by the existing environmental nanoparticles' background concentrations, originating from natural phenomena and anthropogenic activities. Yet another significant process-related source of airborne nanoparticles is frequently ignored: the process-generated nanoparticles (PGNPs), for which it is likely that the potential hazards of PGNPs equal those of MNMs. PGNPs generated at the workplace may even dominate the airborne nanoparticles' number concentration, due to processes used with a frequently more permanent character (and emission). Industrial processes (including conventional processes without any relation to nanotechnology and processing of nanomaterials) may generate air-borne nanoparticles, sometimes up to levels of several millions of particles/cm3. The type of the airborne PGNPs is highly specific for the materials processed, the way of processing, machinery used, temperature etc. Typical sources for the formation of PGNPs at workplaces are heating- and combustion processes, soldering, welding, and fracturing and abrasion activities like sanding, milling, polishing and drilling. But also laser etching and less obvious activities like extrusion and the use of specific electrical equipment may emit nanoparticles in significant amounts.

As long as the hazards of PGNPs and the exposure characteristics are not fully understood a precautionary approach towards risk management and risk communication is indicated. This determines not only responsibilities for the employers, but as well for the original equipment manufacturers, who should inform their clients about the possible hazardous emissions from their machines.

The projects NanoDiode and the Dutch project "exposure registration for working with nanomaterials" strongly focus on these issues.

## Oral free communications | FC5

### Changes in cholesterol homeostasis and acute phase response following pulmonary MWCNT exposure links MWCNT to risk of cardiovascular disease

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Adverse lung effects in rodents following pulmonary exposure to multi-walled carbon nanotubes (MWCNT) are well documented. However, systemic effects are less understood. Prospective epidemiological studies have shown increased cardiovascular disease risk after pulmonary exposure to airborne particles, but the molecular mechanism inducing this risk is not fully clarified. Concerns have been raised that inhalation exposure to MWCNT may increase cardiovascular risk.

We analysed parameters related to cardiovascular disease, including plasma acute phase response (APR) proteins and plas-

ma lipid composition, in female C57BL/6 mice exposed to a single intratracheal instillation of 18, 54 or 162 µg/mouse of small, entangled (CNTSmall, 0.8±0.1 µm in length) or large, thick MWCNT (CNTLarge, 4±0.4 µm in length). Liver tissues and plasma were harvested 1, 3 and 28 days post-exposure. In addition, global hepatic gene expression, hepatic cholesterol content and liver histology were used to assess hepatic effects.

The two MWCNT induced similar systemic responses despite the very different physiochemical properties. APR proteins SAA3 and haptoglobin, as well as plasma total cholesterol and low-density lipoprotein/very low-density lipoprotein, were significantly increased following exposure to both types of MWCNT. Similarly, gene expression analysis revealed perturbation of the same biological processes and pathways in the liver, including the HMG-CoA reductase pathway. Both MWCNT induced similar morphological hepatic changes, and there were a tendency towards greater response following CNTLarge. Acute phase proteins and high systemic cholesterol levels are risk factors for cardiovascular disease and therefore, these results link pulmonary exposure to MWCNT with risk of cardiovascular disease.

## Nickel and nickel oxide nanoparticles cause distinct genotoxic effects in human lung cells

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The different potential of various nickel compounds and particles to induce neoplastic phenotypic changes in somatic cells raises questions about the cyto- and genotoxic mechanisms underlying these phenomena, particularly at nanoscale level where few studies are available. The aim of this study was to perform an in-depth investigation regarding the genotoxicity of Ni and NiO nanoparticles (NPs) as well as $NiCl_2$ following exposure of Beas-2B human lung cells for 48h (1, 5 and 10 µg/mL). Genotoxicity and cytotoxicity were investigated by the comet assay, cytokinesis-block micronucleus cytome assay, colony forming efficiency (CFE) assay and by exploring chromosomal aberrations. We found that NiO-NPs were more cytotoxic compared to Ni-NPs as indicated by a decreased CFE and replication index. Interestingly, Ni-NPs instead increased the replication index in the lowest exposure concentration. NiO-NPs also induced more DNA strand breaks and micronuclei in binucleated cells compared to Ni-NPs. In contrast, Ni-NPs and $NiCl_2$ were more effective in inducing nucleoplasmic bridges and nuclear buds, as well as aneuploidogenic effects (micronuclei in mononucleated cells). Moreover, analysis of chromosomal aberrations revealed a higher capability of NiO-NPs to induce chromatid/chromosome gaps/breaks compared to Ni-NPs and $NiCl_2$ that were more effective in inducing dicentric chromosomes and endoreduplications. Taken together, our results show that Ni and NiO NPs cause distinct genotoxic damage, Ni-NPs being more similar to $NiCl_2$, suggesting differences in the underlying mechanisms and possibly a different carcinogenic potential. Furthermore, exposure to these NPs should be avoided due to the link between the observed genotoxic insults and carcinogenesis.

## In vitro toxicity evaluation of indium tin oxide (ITO) nanoparticles on human lung epithelial A549 cells

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Indium tin oxide (ITO) is mainly used in liquid-crystal displays (LCDs) or plasma displays and mobile phone displays. According to the increased production of ITO, the potential health hazards caused by occupational exposure to this material have attracted much attention. Epidemiology and fatal case studies have demonstrated that inhalation of indium is a potential cause of severe lung diseases. However, the underlying mechanisms of ITO nanoparticles-mediated toxicity remain unclear. The present study was designed to examine the toxic effect of human lung epithelial A549 cells exposed with ITO nanoparticles. Stable ITO medium dispersions were obtained by pre-adsorption and centrifugal fractionation methods and were applied to A549 cells for 6 h, 24 h, 3 days and 7 days. Mitochondrial activity (WST-1 assay), membrane leakage of lactate dehydrogenase (LDH assay), cell proliferation (clonogenic assay), and intracellular reactive oxygen species (ROS) level (DCFH assay) were

assessed. Short-term exposure to ITO nanoparticles showed little adverse effect on the A549 cells, while long-term exposure showed reduced mitochondrial activity and induced leakage of LDH in concentration- and time-dependent manner. The ITO nanoparticles were also found to inhibit the colony forming ability in dose-dependent manner. Further, the ITO nanoparticles induce oxidative stress revealed by the induction of ROS. These results demonstrate that long-term exposure to ITO nanoparticles induce a production of intracellular oxidative stress leading severe cytotoxicity.

## In vivo toxic effects of uncoated zinc oxide nanoparticles

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Zinc oxide nanoparticles (ZnO NPs) are toxic to various organisms, both in vitro and in vivo. In this study, we examined the toxicity of uncoated ZnO NPs (NM-110) administrated by whole-body inhalation to mice. C57BL/6J mice were exposed to three concentrations of ZnO NPs (2.99, 6.68 and 13.52 mg/m3) for 4 days, 4 h/day. DNA damage was assessed by the comet assay in bronchoalveolar lavage (BAL), lung, and white blood cells. Further possible systemic genotoxic effects of ZnO were assessed by the analysis of micronuclei in bone marrow polychromatic erythrocytes. DNA damage showed a dose-dependent increase in BAL cells, and there was also a significant increment in lung and blood cells at the two lower doses. However, ZnO did not induce micronuclei in bone marrow. In addition, ZnO caused pulmonary neutrophilia accompanied by an increased number of macrophages and lymphocytes. Furthermore, an elevated mRNA expression of IL-1 , TNF , IL-6 and IL-13 was observed in the lungs. Histopathological assessment revealed groups of adherent macrophages in alveolar spaces and mitotic cells in the lung tissue. Moreover, markers of oxidative status - level of superoxide dismutase, glutathione peroxidase, and thiobarbituric acid reactive substances (measuring lipid peroxidation) - were increased by ZnO in a dose-dependent manner in BAL, and statistically significant differences were also observed in plasma at the highest dose. Our findings suggest that inhalation exposure of mice to ZnO NPs induces inflammation and genotoxic effects in the lungs and a systemic oxidative stress (Funded by the EU FP-7 MARINA, Grant Agreement No.263215).

## Studying nanoparticle translocation and behaviour at the human placental barrier using ex vivo and advanced in vitro model systems

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With the growing use of nanotechnology, the placenta is likely to come into contact with nanoparticles (NPs) either accidentally through exposure or intentionally in the case of nanomedical applications. Therefore studies dealing with the placental translocation and effects of NPs are of utmost importance in particular if considering the significant knowledge gap in this field of research.

We investigated placental NP translocation by using the ex vivo human placental perfusion model. For more mechanistic studies requiring higher throughput, we are developing two novel in vitro placenta systems.

We showed a size-dependent transfer of polystyrene NPs across the human placenta. Moreover, bidirectional transport studies demonstrated an increased transfer of PS beads in reverse (fetal to maternal direction) perfusions and an accumulation of PS beads in the syncytiotrophoblast layer of the placental tissue. Establishment of novel in vitro placenta models is ongoing: 1) we have set up co-cultures of endothelial and trophoblast cells on transwell inserts that will be inserted in a perfusion chamber to mimic maternal/fetal circulation 2) we obtained stable, organized 3D-microspheres from placental fibroblasts and trophoblast cells using a hanging-drop approach.

Our ex vivo perfusion studies indicate that the syncytiotrophoblast is the key player in regulating transplacental NP transfer, and that the underlying mechanism is not based on passive diffusion but involves active, energy-dependent translocation pathways. Complementary mechanistic studies using a large variety of NPs in advanced in vitro models will be required to support the safe use of NPs and the design of novel nanomedical therapies in pregnancy.

# Oral free communications | FC6

### Release of nanosized pigments from paints under wet use phase solicitations

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Nanoparticles are incorporated in paints to improve their mechanical properties, their UV resistance and to control their colour. This emerging industry takes into consideration a responsible development of its products regarding human and environment safety (eco-design). This concern requires a careful control of the nanoparticles added in consumer products throughout their entire life cycle. Since toxicity is still in debate for most nanoparticles, some studies now focus on the "non-releaseability" of nanomaterials.

This project is based on the identification of the release mechanisms of nanoparticles from paints (wet route) with special focus on the influence of the chemical and physical properties of the particles on the rate of release. The final objective is to improve the paints formulations. Here, nanoparticles (TiO2, SiO2, Carbon Black and others organic pigments) were added to the formulation as pigments. In order to simulate outdoor aging and washability during the life cycle of the products, painted panels were exposed to accelerated weathering and to water. The conditions of the tests followed the ISO norms of exposure to laboratory light sources and the determination of wet-scrub resistance / cleanability of paints. The protocol involved to analyze the effect of exposure on nanomaterial surfaces as well as possible nanoparticles contamination in runoff waters. These samples of leachate water were analyzed by Dynamic light scattering (DLS) and by an analytic chain: a field flow fractionation (FFF) coupled to light scattering, to refractometry and to Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

## Workplace measurements in the facility producing CNTs

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Occupational exposure to engineered nanomaterial (ENM) has been under intensive study in recent years due to the postulated negative health effects of some nanomaterials [1]. Thus, measurement data on occupational exposure in real working environments is seen important. We measured occupational exposure to engineered nanomaterial in a workplace where carbon nanotubes (CNT) were produced [2]. Particle size distribution and total concentration were measured for the size range 2.5 nm – 10 µm continuously using different on-line measurement instruments. The gas phase compounds i.e. CO and CO2 were sampled continuously with direct reading instruments as well. During the measurement campaign several particle samples were collected for further analysis with transmission electron microscopy (TEM).

The work place measurement campaign was implemented as a long term resulting over a week of continuous data. During the campaign we were able to distinguish the work phases with possible release of CNTs. Measurement results were used to assess workers' exposure to CNTs. Along with the measurement results the ability of different measurement instruments to detect the release of CNTs into the workplace breathing air is discussed. The risk management techniques suitable in this case are also considered.

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## Identifying, assessing and controlling nanoparticle exposures among U.S. construction workers

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The European construction industry has generally been ahead of the U.S. in the use of nano-enabled materials, but the nonprofit organization CPWR, with NIOSH funding, has identified over 400 potentially nano-enabled construction products, of which three-fourths appear to be available in the U.S. As part of a 5-year nanotechnology effort, CPWR has posted these products on the electronic Library of Construction Occupational Safety and Health (http://www.elcosh.org), which receives 30,000 visits each month. Over half of the products in the database are coatings and 26 different nano-objects have been identified as additives to the various products. Internationally, very little exposure monitoring has been conducted for nano in construction. Evaluating risks in construction is complicated by the constantly changing work environment, the presence of multiple contractors, regular bystander exposures and the possibility that the most serious exposures may not occur until a building is demolished decades later. The authors conducted written surveys of 79 union trainers from 15 different trades and found almost half were unaware that nanotechnology has been applied to construction materials. This is similar to results from a European survey. CPWR also conducted air samples while photocatalytic roofing tiles containing nano-TiO2 were cut with a masonry saw. Samples were collected with no controls and also with local exhaust ventilation (LEV) from a HEPA vacuum attached to the saw. Non-agglomerated TiO2 particles were released, but none of the exposure measurements exceeded the NIOSH Recommended Exposure Limit for ultrafine TiO2. Use of LEV resulted in a significant reduction in mean concentration of respirable dust when compared to no LEV. Similar significant reductions in median particle counts were seen when an electric grinder was used on a mortar compound containing nano calcium silicate.

## Comparing workers measured dust exposure with predicted exposures using a NF/FF model, NanoSafer, and the ART exposure assessment tools

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The REACH requires that manufacturers or importers within European Union must estimate human exposure by all routes for each potential exposure scenario which will then be used in risk assessment. To fulfill this regulation several advanced exposure assessment models were developed. These have shown to work well for solvents but their performances to predict particle exposure have not been tested in real work environment.

Here we measured concentrations during pouring of paint pigments/fillers from small bags (25 kg) and big bags (500 kg) in a paint factory which were carried out within 1 m and 8 meters from a mixing station [1]. The pigments/fillers dustiness indices were characterized by using the down-scaled EN15051 dustiness drum [2]. Dustiness indices were used to calculate the dusts emission rates used in the tools by taking into account modifying factors [3]. The measured concentrations were compared with concentrations predicted with the Near Field/Far Field (NF/FF) model [4], NanoSafer (http://nanosafer.i-bar.dk/), and the ART (https://www.advancedreachtool.com).

The NF/FF model comparison showed that a handling energy value deviated significantly from previously assigned values [3]. The ART tool was found to overestimate ~5 times the exposure concentration. It was shown that the emissions from powder handling still need to be studied in well controlled environments.

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## From comparison tests to recommendations in standardisation for counting nanoparticles by using CPCs

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A standard dedicated for the use of condensation particle counters to determine workplace exposure by measuring the number concentration of nanoparticles and their aggregates/agglomerates is still missing. The mandate M/461 from the European Commission for the support of regulation in nanotechnology therefore includes a dedicated project in CEN/TC137. The CEN standard to be developed shall give advice for occupational hygienists and researchers in their task of assessing exposure to nanoparticles.

In order to enlarge the knowledge of the behavior of CPCs, a pre-normative research campaign was conducted by 12 partners, mostly from European institutes for occupational safety. The use of CPCs under several conditions was subject to comparison tests in the Nano Test Center of the IGF in Dortmund. In total 12 different types of CPCs and 35 individual instruments had been compared, handheld and stationary, alcohol and water based. 28 different test aerosols were generated by a flame generator (NaCl, ZnO), spark generator (Cu, C) and a droplet generator/atomizer (DEHS, NaCl). A wide range of concentrations (14 000 to 760 000 cm-3), particle mobility diameters (5 to 260 nm) was supplied to the instruments that were located in a cham-

ber of 3x3x2.5 $m^3$. Questions of dependency on concentration, particle diameter, chemical composition, resp. hygroscopicity, and particle morphology in comparison with the statements of the manufacturers were in the focus of interest. Deviations up to approximately 30 % from a reference seem to be reasonable in the specified operational ranges. A missing calibration may contribute to larger discrepancies.

# Oral free communications | FC7

### Genetic changes and circulating protein levels in Danio rerio exposed to gold nanoparticles (nAu) in aquatic media

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Aquatic toxicology of nAu using a combination of acute and chronic ecotoxicological bioassays using Danio rerio as a model organism was performed. Range finding exposure tests were done to determine the LC50 of nAu in D. rerio using standard OECD protocols where ionic gold and solvent controls were also included. Adult zebrafish were exposed to nAu for 96 hours at a concentration range of 5mg/l to 45mg/l. Grouped liver samples were stored in RNA later and were used for DNA microarray as well as RT-PCR to determine changes in gene expression. Whole body samples were stored in respective buffers for biomarker analysis. The results obtained from scanning the gene chip gave an indication of events in the cell based on 15 618 genes being regulated. The gene ontology was further investigated using ArrayStar to show pathway interactions as well as clustering of genes. Pathways were then looked at and the following genes were focused on and used for primer design and RT-PCR: Cat, Sod, MT1, MT2, gpx1a1, Cyp1a, Cpy1c1, Cyp11a, Cyp17a1, Cyp19a1 and Bactin, which was used as endogenous control. The up or down regulation of these genes were then further confirmed by looking at biomarker responses in whole body organisms to explore whether genetic changes were influential of circulating protein levels after a 96 hour period. A bimodal response was observed in the various biomarkers analysed and cytochrome P450 showed increased activity when compared to control while the ionic gold exposure concentrations showed none.

### FullereneC60 loading interferes of the growth and emergence rate of the Midge Chironomus riparius

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The suitability of test methods for use with nanomaterials is a topic of significant research interest. There are several uncertainties when assessing nanomaterials, such as fullerene(nC60), potential toxicity to aquatic invertebrates because of their unique chemical properties which substantially differ from those of hydrophobic organic chemicals and dissolved metals. Aims of this study were to investigate the method for measuring the body residues on Chironomus riparius and to study if sediment assosiated fullerene had an impact on larval development and emergence rate. A benthic invertebrate C. riparius larvae were exposed to nC60 with two different exposure methods; 1) masses in the sediment top layer creating an environmentally realistic method and thus simulating a sensitive exposure route for C. riparius feeding habits and 2) using fullerene spiked sediment which is commonly used method in ecotoxicology. Body residues after acute and chronic exposures were analysed and larval growth and development rate assessed. Body residues were lower in the exposure method 1 after 15 d exposure than after 10 d which may stem for changing in behaviour of larval feeding habits during the development time or possibly after 10 d time larvae have reached steady state. Using the exposure method 2, larval growth and emergence rate

were affected by fullerenes. Findings indicated that presence of fullerene interferes organisms even at the lowest tested (method 2, 0.5 mg/kg) concentration. Overall, these results pose that there is possible ecotoxicity towards benthic organisms that fullereneC60 may cause leading changes in ecotoxic parameters used here.

## NanoMILE at the halfway point: mechanistic insights and progress towards a grouping and classification framework

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The overarching objective of the EU FP7 funded large collaborative project NanoMILE is to formulate an intelligent and powerful paradigm for the mode(s) of interaction between manufactured Nanomaterials (MNMs) and organisms or the environment, to allow the development of a single framework for the classification of nanomaterial safety according to their biological impacts and to create a universally applicable framework for nanosafety. The project started in March 2013 and by its halfway point (March 2015) has made several notable achievements including: publication of the NanoMILE consortium's initial framework for classification of nanomaterials; development of a classification framework for environmental transformation reactions impacting nanomaterials and whether these reduce or increase the diversity of particles to be tested; identification of the of body target tissues accumulating nanomaterials across a variety of ecological species; and development of heatmaps to classify nanomaterial toxicity based on high content and high through single cells. Our industrial partners have been actively involved in consortium advances, including development of libraries of systematically varied nanomaterials (e.g. systematic reduction of redox activity of ceria nanomaterials for use in vitro and in vivo) by Promethean Particles; and development of an automated camera focus adaption for NanoSIGHT by Malvern Instruments to enable time-resolved characterization of nanoparticle dispersion stability and sedimentation and/or agglomeration. In vivo translocation studies with 14C labeled carbon nanotubes and the time course of lung retention and toxicity of inhaled ceria nanoparticles have also been assessed. For more details on project outputs to date, please see the project website: www.nanomile.eu.

## Standardized in vitro high-throughput screening analyses serve efficiently for rapid ranking of nanomaterials toxicity under diverse testing protocols

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Engineering of nanomaterials (ENMs) is a key enabling technology on the basis of the future incorporation of a safe-by-design evaluation protocol. High-throughput screening (HTS) in vitro would potentially enable toxicity ranking of the many ENMs to be generated under such a strategy. We report a standardized protocol for a 384-well HTS-based safety analysis of ENMs. The BEAS-2B human lung epithelial cell line and the Promega's CellTiter-Glo assay was applied for analysis of cellular ATP content as a surrogate measure for alterations in cell numbers and viability. Established reference ENMs, including oxide forms of iron, titanium, zinc and copper, and so far untested, customer-based ENMs, demonstrated dose-dependent toxicity over a wide range of concentrations, indicating manifold differences in potency among different ENMs. Factors like the dispersion protocol, storage time of the ENM dispersions, cell density, and culture conditions were found to influence the testing results. Interestingly, we noted that a particular dispersion protocol could variably act to increase, decrease or not affect the ENM toxicity effect relative other dispersion protocols. The tested ENMs as such did not influence the toxicity readout. Quantification of cell surface areas by microscopic imaging under time lapse demonstrated the feasibility of simultaneous screening for morphological toxicity. Including studies of customer-produced ENMs of variable toxic potency, we demonstrate a standardized protocol that permits rapid sorting of possible influences of testing variables in ENM safety testing. The results argue for the value of proactive ENM safety evaluation under a safe-by-design concept.

### High content imaging reveals cell type-specific nanotoxicity

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Nanotechnology is advancing rapidly, as reflected by the vast increase in newly engineered nanomaterials and innovative applications. In contrast, nanosafety lags behind as mechanistic insights into how nanoparticles (NP) cause toxicity are largely lacking. To tackle this issue, standardization of nanotoxicity assessment is imperative. In this regard, several groups focus on NP characterization in physiologically relevant media, assay interference etc. In our opinion, the cell type used for in vitro studies is of equal importance. Especially since the use of cell lines can be questioned due to their altered metabolism and apoptotic balance. Hence, we evaluated whether a cell line can be used as a model for in vitro nanotoxicological evaluations. To this end, we studied NP induced effects in a cancer cell line, an immortalized cell line and neural stem cells derived from both humans and mice. First, acute toxicity induced by AuNP, AgNP and IONP was determined. We observed a concentration dependent decrease in viability in nearly every cell type-NP combination. The degree of acute toxicity could furthermore be correlated to the core material, as the AuNP and IONP caused most and least toxicity, respectively. Next, we examined sublethal effects generated by the IONP via a high content imaging approach where we probed for induction of reactive oxygen species, effects on mitochondrial membrane potential, intracellular calcium content and cell morphology. We discovered that IONP exposure evoked a cell type specific combination of effects that could not be observed in another cell type.These data clearly reveal cell type specific toxicity profiles and indicate that a cell line cannot simply be used to model nanotoxicity. In conclusion, this study underscores the importance of thoughtful cell type selection for nanotoxicity studies based on the intended NP application and/or in vivo exposure.

## Oral free communications | FC8

### Risk assessment of inhalation exposure to engineered nanomaterials

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In toxicology, quantitative risk assessment is currently based on exposure assessment and hazard assessment with in vivo studies. However, due to nanotechnology, there is increasing need for faster and predictive tools for assessing ENM hazards. For this purpose, methods have been developed to estimate the biological effects of the deposited dose, using:

1. In vivo studies to estimate dose-response for specific toxicological endpoints [1].
2. In vitro studies together with a cell dosimetry model to estimate risk-range [2].
3. Equivalent dose to estimate relative risk [3].

The equivalent dose is the deposited dose weighted by factors that takes into account physico-chemical properties of the nanoparticles effecting on the biological response. Such a method is based on grouping of nanomaterials [4] and is applied e.g. in risk assessment modelling and control banding tools.

Here, we show how the quantitative risk of workers exposed to ENMs and nanoparticles in different occupational settings could be assessed. Source specific exposures and inhaled dose rates were assessed [5]. Inhaled dose rates were calculated and the workers' risks were estimated by using the listed methods. This was performed for workers exposed to nTiO2 using (in vivo), nanodiamonds (in vitro), and incidental nanoparticles (equivalent dose).

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## A 'multiple perspective' framework for the grouping of nanomaterials

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The grouping of substances serves to streamline testing for regulatory purposes. Currently, specific regulatory frameworks for the grouping of nanomaterials are unavailable. Notwithstanding, this topic is addressed in different publications, and preliminary guidance is provided in the context of substance-related legislation or the occupational setting. A review of available concepts conducted by the European Centre for Ecotoxicology and Toxicology of Chemicals Task Force on the Grouping of Nanomaterials (ECETOC Nano TF) came to the conclusion that all approaches for the grouping of nanomaterials for human health risk assessment go beyond the determination of structure-activity relationships. They are founded on different aspects of a nanomaterial's life cycle, including its material properties and biophysical interactions, specific types of use and exposure, uptake and kinetics, and potential early and apical biological effects. None of the available grouping concepts takes into account all aspects of the nanomaterial's life cycle. To promote application of nanomaterial grouping in practice, the ECETOC Nano TF is putting forward a comprehensive 'multiple perspective' framework for the grouping of nanomaterials. This framework allows for a combined, sequential grouping of nanomaterials by all aspects of their life cycles that are relevant for risk assessment, starting from their use, to release, uptake and finally the toxic effects of the material in different life-stages of the product. The 'multiple perspective' framework can be applied to determine needs to address different exposure scenarios, to prioritize potential risks, to apply read-across techniques, to justify waiving of testing, or to define criteria for testing strategies.

## The collective protection against nano-objects

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The collective protection against nano-objects can be assured by the application of effective ventilation systems equipped with appropriate air filters.

This work presents the results of the testing efficiency of different alternatives for collective protection systems, i.e. included both:
- investigation of parameters directly connected with efficiency of ventilation: general ventilation (exchange rate) and local ventilation (efficiency of LEVs),
- investigation of parameters connected with the air movement in the room equipped with general and/or local ventilations – determination of concentrations and mean diameter of particles and air parameters (temperature, humidity and velocity) in different places in the room, in the breathing zone of workers or close to the source of nano-objects and in another five points in which accumulation of nano-objects were expected.

It was confirmed that only in the room with positive pressure ventilation and when works were conducted in the glove box, particles from the processes were not transferred to the room air. In the same room and when works were conducted in the fume cupboard, particles from the processes were transferred to the room air, mainly on the places located on direction of particles emission. In all another options of ventilation in tested rooms, nano-size particles emitted during processes were transferred to the room air, even in the areas located far from places of processes (up to about 17 m). Switched-on local ventilation systems (when negative pressure ventilation was occurred in the room) results in decrease the concentrations of particles emitted during processes with nanomaterials, but effects on increase of the background particle concentrations as results of transferring to the air particles from the dust settled on the surfaces.

This work presents the results of the projects: SCAFFOLD (GA 280535) and II.P.02 (CIOP-PIB).

## Level of control self-assessment method for handling of engineered nanomaterials

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Comprehensive risk assessment for ENMs faces serious challenges in the current uncertainty of nanomaterial health effects and the lack of the official occupational exposure limit (OEL) values. Therefore, it is commonly recommended that the level of control should follow the precautionary principles. Currently, several documents of good practises and recommended control measures for working with nanomaterials are available.

In this study, a new standpoint for evaluating the safety of nanomaterial handling is presented. This study was performed as a part of FP7 MARINA (Managing Risks of Nanomaterials) project. The main idea of the approach is to identify the current control level and thereby find control measures which need to be improved in order to achieve best practices in the absence of OELs. The base for this comes from an idea, that every workplace should fulfil at least a certain minimum level for ENM control measures, similar to the handling of all other chemicals. In the first step the use of nanomaterial is evaluated by simple categorization to different ENM states and treatments, resulting in appropriate control level (BASE, ADVANCED or SPECIAL-levels). At this appropriate control level, the checklist gives recommendations for control measures to be utilized. The good practises and recommended control measures found in the literature were used to prepare the checklists. The most important outcome of this approach is that companies should find out a tangible basis for the improvement of the safe handling of ENMs.

## Penetration of engineered nanoparticles through nitrile rubber gloves

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Engineered nanoparticles (ENP) are studied and employed in the formulation of several commercial products. An increasing number of scientists and workers are handling them. Moreover, recent studies have confirmed that the skin is not an impervious membrane against nanoparticles. According to these findings, many Health & Safety agencies have recommended the use of protective gloves against chemicals as a mean of protection during the handling of ENP.

Until recently, few studies have examined the effectiveness of protective gloves against ENP. In these early works, ENP were in direct contact with glove materials subjected to static deformation. However, the published results were inconclusive. . Additional studies have focused on the penetration of titanium dioxide in powder or dispersed form through protective gloves under conditions simulating occupational use.

This communication discusses the current state-of-the-art on the efficiency of common protective gloves against ENP. Results obtained with gold nanoparticles in water (nAu) and two types of nitrile rubber gloves will be presented. For this work, 50%-biaxial dynamic deformations simulating the flexing of the hand were applied to the nitrile rubber gloves during their exposure to nAu. ENP penetration through the gloves was determined by measuring gold concentration in a sampling solution having a chemical composition similar to human sweat.

Biaxial deformations and sampling solution affect the mechanical and physical properties of the gloves tested which could modify their efficiency against ENP. Pending further investigations, great care must be taken in selecting protective gloves for the handling of ENP.

# POSTER SESSION 1
# Monday 13 April 2015

## THEME : SAFE NANOMATERIALS AND THEIR APPLICATIONS

### 1. Engineered nanomaterial interactions with ecological exudates - potential for impact on complex ecosystem signalling pathways

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Nanoparticles (NPs) have a high surface energy that they seek to lower by binding to available biomolecules from their surroundings such as components of product formulations, proteins or lipids in living systems, natural organic matter (NOM) components of water or soil or potentially exuded and secreted biomolecules in complex ecosystems. Formation of a protein corona around NPs is a ubiquitous phenomenon that occurs instantaneously upon contact with available macromolecules. Most research to date has focussed on the interactions of NPs with blood proteins (human or animal sera) or lung surfactant proteins, to correlate corona composition with NP uptake and impacts on living systems. Environmental interactions to date have focussed on NP-NOM interaction studies, primarily assessing the impact of the humic substances on particle stability/bioavailability. Much less work has investigated the potential for NPs to bind the exuded biomolecules central to much of the plant and microorganism world, where secretion of biomolecules can be a defensive response to repel insect attack, or an offensive habit to repel other incompatible or competitive plants. Initial experimental work related to the “ecocorona” will be presented, focussing on NP interactions with exopolysaccharides and proteins in the exudate from biofilms and how this correlates with the biofilm responses to the presence of NPs, and from daphnia magna, and on the interactions of NPs with conditioned daphnia magna media and the role of the absorbed ecocorona in mediating NP uptake by and impacts on daphnia. The impacts of exudate binding on NP transformation will also be discussed.

### 2. Acute toxicity of silver nanoparticle for zebrafish (Danio rerio embryos): effect of nanoparticle stabilization and physicochemical properties

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Recently nanoparticles have got wide application in technology, industry and science. A lot of companies already integrate nanomaterials in their products. Although the nanotechnology field is growing rapidly, the potential harmful effects of nanomaterials on human’s health or the environment have not yet been identified. Their activity in environment is still unclear. It is very essential to figure out possible toxic effect of nanoparticles on biocoenosis and type of mechanism of interaction with live organism. Understanding mechanism of toxicity nanomaterials might be very helpful for making forecast about safety of further increasing of productivity nano-sized materials. Within our study we investigate influence of different type of stabilizer on physicochemical properties, stability and behavior of silver nanoparticles. We observed how change activity of NPs in time and that happened with silver NPs during experimental time. Contribution of stabilizer and particles themselves in toxicity for Danio rerio were analyzed. Obtained data were compared with antibacterial properties of nanosilver.

## 4. NanoDiode - Developing innovative outreach and dialogue on responsible nanotechnologies in EU civil society

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NanoDiode is a European project focused on the engagement of stakeholders in the governance of nanotechnologies. It involves activities along the innovation value chain that will give citizens and civil society organisations (CSOs) a voice in both the development of nanotechnologies and in determining what the safe use of nanomaterials is, so as to support the effective governance of nanotechnologies and encourage its responsible research and innovation.

Dialogue and outreach activities:
The introduction of new technologies is generally accompanied by uncertainties about the claimed benefits and risks. The same holds true for nanotechnologies, which are being developed as potential tools for use in almost all existing areas.

As such it raises many questions: Do we need the new technology? Can we benefit from it? Can we control the risks? Can we live with the uncertainties? Can we influence the direction of research and innovation? These and many more questions on the edge of innovation and ethics will be asked by NanoDiode.

## 5. Biodegradability, compostability and safety of cellulose nanofibrils (CNF) and CNF-based products

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Cellulose nanomaterials have unique properties that make them potential for numerous applications. One of their most promising applications is transparent cellulose films with excellent strength, density and smoothness. Cellulose films could be utilized as gas barriers in packaging, substrates for printed electronics, in diagnostics or even as electronic displays instead of oil-based plastic films. From the safety point of view, the advantages of cellulose nanomaterials have been argued to be their benign nature towards humans and the environment as well as their biodegradability.

Results on the biodegradability of different grades of cellulose nanofibrils (CNF) and their products, namely CNF films and papers coated with CNF are presented in this paper. In addition, results on the toxicity of cellulose nanofibrils to humans as well as ecotoxicity during biodegradation in the composting environment are reported. The results of biodegradability tests (OECD 301B) of two different grades of CNF indicate that their biodegradability is dependent on the fibrillation degree, the finer grade degrading faster than the coarser grade. The biodegradability test (EN 14046) of CNF films and papers containing CNF showed that all the CNF products tested were biodegradable according to the requirements set in the standard (EN 13432). In the composting test (EN 14045) disintegration of CNF products was observed in the composting environment with no acute ecotoxicity during biodegradation. Regarding toxicity to humans, some indication of cytotoxicity was observed for nano-scale cellulose fibrils. However, all the other toxicity tests results, including in vivo tests with Nematode, showed no sign of toxicity.

## 6. Synthesis of nanoparticles coated with levan from Pseudomonas syringae and their beneficial in vitro effects on bacteria and mammalian cells.

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Fructans such as levan and inulin have a large potential in biotechnology due to their prebiotic and immunostimulating effects. We have previously characterized the levansucrase Lsc3 of Pseudomonas syringae DC3000 as efficient and stable levan producer. In this study we conducted the large-scale synthesis of levan and tested it for the potential to coat and stabilize various mineral-based nanoparticles (NPs) to be potentially used as food supplement. In parallel, we studied the effects of levan and levan-coated NPs on bacteria and mammalian cells in vitro.

Using transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS) and zeta potential measurements we showed that $Fe_3O_4$, $Co_3O_4$ and Se NPs can be stably coated with levan. DLS measurement showed that levan-stabilized NPs were more stable in bacterial and cell culture media compared with uncoated NPs.

Bacterial assays showed that addition of 625 mg/l of levan to bacterial culture medium significantly improved the growth of beneficial gut bacteria Lactobacillus casei, whereas had little effect on potential human pathogen Escherichia coli. In addition, levan increased the metabolic activity of Caco2 cells – in vitro model for intestinal epithelial cells. The coating of Co3O4 and Se NPs with levan significantly reduced their toxicity to Caco2 cells. We conclude that levan is a biocompatible coating agent that both disperses NPs and has selective beneficial effects on bacterial and human cells in vitro.

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## 8. Efficient image analysis technique to study the primary nanoparticle size distribution from TEM micrographs

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One of the big missions of today's health and safety research is to get reliable information on the effects of engineered nanoparticles in living organisms. A complete study of occupational exposure and toxicity requires an in-depth nanoparticle characterization to ensure reproducibility of the results and to understand biological effects of nanoparticles. However, the characterization of nanoparticles is challenging due to their multiplicity, multiformity and agglomeration. One of the most effective methods to characterize general nanoparticle characteristics from the bulk nanomaterial is transmission electron microscopy (TEM). TEM analysis is performed for nanoparticle samples to provide raw data, such as primary particle size, morphology of particles and agglomerates as well as composition and phase structure, for further toxicological studies. Analysis is needed also to confirm the characteristics of commercial nanoparticles. In order to carry out reliable primary particle size analysis, it is necessary to measure a large number of separate particles and thus the use of efficient image analysis system is desirable. In this study we present a novel image analysis technique based on active contour algorithm applied to the distance transformation of TEM image. This technique can measure large numbers of nanoparticles fast and fractionate nanoparticles from TEM micrographs in which particles are overlapping or contacting each other, which is not possible with commercial image analysis programs. The implementation also allows manual editing of the result of automatic segmentation. The technique proved to be much easier and less labour-intensive method to verify primary particle size distributions from easily agglomerating nanopowders than commonly used manual measuring procedures.

## 9. Single particle analysis of nanomaterials with agilent ICP-MS

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Nanoparticles (NPs) are defined as ultrafine particles with one dimension between 1–100 nm [1]. Because of their small size, they have a very large surface area relative to their weight, so they often react quite differently from a bulk solid or dissolved material of the same composition. For this reason they may offer novel and interesting properties for a broad range of applications. Current and potential applications for NPs range from food additives, cosmetics and pharmaceuticals, to biocidal packaging, fuel cell technology and electronics. But while their use is constantly increasing, questions and concerns have been raised about their safety and their health impact. For this reason, there is an urgent need to develop analytical methods

that are suitable for the particular evaluation of NPs.

An interesting approach for the characterization of NPs has been developed by Degueldre et al. using ICP-MS [2]. In the present work the analysis of silver NPs has been carried out using the Agilent 7900 in SP-ICP-MS mode. The method allows the determination of the nanoparticles mass concentration but also the evaluation of their median size and size distribution.

1. ISO TS 80004-1:2010: Nanotechnologies - Vocabulary - Part 1: Core terms
2. Degueldre S., Favarger P.-Y., Bitea C., (2004) Analytical Chimicta Acta, 518: 137-142

## THEME : EXPOSURE: DETECTION TECHNOLOGIES, ASSESSMENT AND MODELING

### 11. Occupational exposure to nano-$TiO_2$ and to nano-$SiO_2$ in construction: the challenge of background discrimination and selective detection of target analytes

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The construction sector is increasingly incorporating manufactured nanomaterials (MNMs) in products such as cements, concrete or insulations. Widespread applications are depolluting mortars (nano-TIO2) and self-compacting concrete (nano-SiO2). This work has been done in the framework of project SCAFFOLD (GA 280535), aiming at the management of potential risks arising from MNMs in construction. The goal is to measure the occupational exposure to nano-TiO2 and nano-SiO2 in real-life application along their life-cycle.

The experimental strategy encompasses a series of real-time and time-integrated aerosol analysis techniques. The characterization of the released particles has been done using selective analytic techniques jointly with SEM/EDX analysis. The challenge is to ascertain the exposure to nano-TiO2 and nano-SiO2 in scenaria where exposure to different kinds of dust is extremely common and both background and target aerosol undergo significant changes along a sequence of operative tasks. Measurements have been performed at both pilot and industrial scale in similar conditions to those of real practice.

Results will be presented about the release dynamics along a sequence of tasks performed with/without MNMs (e.g. mixing, machining) as well as data on exposure to the target analytes, namely additions of nano-TiO2 and nano-SiO2. Nano-TiO2 results are expressed on mass concentration basis. Measurements of amorphous nano-SiO2 are currently being performed by Raman spectroscopy. This work will provide data on exposure to MNMs jointly with issues about measurement methods as arisen from experience.

## 12. Exposure to airborne nanoparticles from laser processes applied to the ceramic industry

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Evidence that workers in the ceramic industry are exposed to harmful airborne particles has been increasing, especially during ceramic tile sintering in furnaces [1, 2]. Laser technology, coupled to conventional furnaces, is currently starting to be used for the improvement of surface properties of tiles. However, nanoparticle emissions during laser treatments have so far never been assessed. This work aimed to identify and quantify particle emissions (with special interest in nanoparticles; Dp < 50 nm) from tile sintering applied to two types of tiles (porcelain and red clay). Particle number, mass concentrations, alveolar lung deposited surface area (LDSA) concentration in the 5 - 20000 nm size range were monitored in the emission source and worker breathing zone. Samples for the analysis of major and trace elements by means of ICP-AES and ICP-MS and for the particle characterization by means of TEM were also collected.

Results from this study evidenced high worker exposures to particle number (up to N = 2.3 x 10^6 parts. cm-3; LDSA = 2.3 x 10^3 µm2 cm-3) in potentially harmful particle diameters (13 - 27 nm). When emissions were transported from the source toward the breathing zone, particle number, mass and LDSA decreased in comparison to those measured at the emission source but remained at high levels.

Concentrations of potentially health relevant metals such as Zn, Pb, Cu, Cr, As and TI were found in particles < 250 nm. This work evidences the risk of worker exposure to nanoparticles and raises a need to develop mitigation approaches.

References:
1. Monfort, E., et al., Applied Clay Science, 2008. 38(3–4): p. 250-258.
2. Voliotis, A., et al., Environ Sci Process Impacts, 2014. 16(6): p. 1489-94.

## 13. The potential for nanoparticle release during demolition and recycling in the construction industry

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The use of nanomaterials in construction is increasing; it has been predicted that they will be present in 50% of building products by 2025. However, the potential for nanoparticle release from these products at their end-of-life is unclear. This study has identified common demolition and recycling activities. Tests which replicate these in a laboratory environment are being used to assess their impact on a variety of nano-enabled building products.

The processes used during demolition and recycling are, by definition, highly destructive, creating a potential for nanoparticle release. The mechanisms involved include cutting, tearing, burning, crushing, melting, drilling, and the use of explosives. Some parts of these processes involve close contact between workers and product. This occurs during ‘soft strip’ when the interior fittings and fabric of a building are removed with hand tools to expose the main structure. There is further close-contact exposure during recycling when hand sorting is used to separate out or dismantle different materials. Others processes are more aggressive and might create risk across a wider area. The health risks are likely to be increased if long-term weathering, such as UV exposure, has deteriorated the matrix which initially secured the particles. The use of carbon nanotubes, which may have asbestos-like effects, is of particular concern. Products containing these are only just being introduced to the construction market. Other materials such as silica fume have been in use for many years and appear to present lower risks, but research work is ongoing in order to investigate this definitively.

## 14. Nanoparticle emissions from ceramic industrial processes

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Dry milling and laser sintering of tiles are energy-intensive processes in ceramic processing; the former is a widespread technique whereas the latter is emerging. Such high-energy processes (thermal and mechanical) have the potential to generate fugitive nanoparticle emissions, which may strongly impact worker exposure. However, to date nanoparticle formation and emission processes from ceramic industrial activities have received little attention in the scientific literature. This work aimed to identify and quantify ultrafine and nanoparticle emissions from these processes in industrial settings, as well as to understand the driving factors influencing emissions.

Real-time and size-resolved particle number, mass, diameter, and surface area were determined (5 nm-20 µm) in two diferent industrial pilot plants. Samples were collected for chemical and morphological characterisation (ICP-AES, ICP-MS, TEM). During dry milling, particle concentrations and size distributions were strongly dependent on the raw material composition (grain size, porosity). Material #1 milling resulted in the highest particle emissions (1.3 x 10^5 particles/cm3), when compared to Material #2. During Material #1 milling 99.6% of new particle emissions in terms of number was <30 nm in diameter, while during Material #2 milling 86% of the particles was > 100 nm.

In the case of laser sintering of ceramic tiles, high nanoparticle emissions were detected (mean 1-minute concentration reaching 9.7 x 10^5 particles/cm3, over 1.5 hour periods), with potentially harmful particle diameters of 18 nm. New particle formation events were detected, in addition to nanoparticle emissions.

This study raises a need to further charcterise nanoparticle emission mechanisms in high-energy industrial processes.

## 16. Challenges in detection of ENPs as hot gas aerosols

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Epidemiological studies suggest that adverse health effects are hardly caused by the inhaled mass of particles but rather by the number of particles. High concentrations of Anthropogenic Nano Particles (ANP) can be emitted in principal by several kind of combustion processes. These ANP consist mainly of elemental and organic carbon. The primary emitted gaseous components are volatile organic carbons (VOC) that will be converted by chemical reactions and condensation processes to soot particles. Beside emissions of soot particles also several industrial processes are emitting hot gas vapors and engineered ANP (ENP) as metal oxides. These particles might also cause harmful health effects and should be observed and controlled. The measuring of ENP at hot gas applications requires first a special sampling procedure that should not change particle sizes or number concentrations and should also avoid any kind of water condensation processes as well as losses of particles. The second challenging step is the detection with suitable aerosol measurement principles as electrical or optical detection methods. A valuable detection of ENP requires not only the quantification of the ENP number concentration but in addition the determination of number and mass distributions depending on aerosol sizes. The company GRIMM has developed cutting edge sampling and measurement techniques for the precise detection of ENP at hot gas conditions. Depending on gas temperatures, particle sizes and concentrations different techniques can be applied. The ESS (emission sampling system) is a combination of a hot gas dilution sampling probe with a scanning mobility particle sizer (SMPS). The latest development is the µ-hot gas sampling probe, that allows the hot gas detection of ENP and the agglomeration of ENP (up to several µm) simultaneously. These measurement techniques and interesting applications will be presented.

## 17. Design of an exposure chamber for the evaluation of personal samplers

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According to NIOSH, there are more than 400,000 workers worldwide that are exposed to nanoparticles. There is no standardized method to assess workers' exposure to nanoparticles. Personal samplers are generally utilized for the collection of aerosols present in the breathing zone of the worker. Particle properties such as size, size distribution, aspect ratio, and surface properties are important in terms of the efficiency of a sampler. In order to reduce measurement errors and obtain accurate results, one should have an understanding of the efficiency of the sampler. This is normally done by exposing particles with known properties to a sampler and then evaluating the sampler efficiency by analyzing the collected particles. Therefore to evaluate the performance of a sampler, there is a need for a chamber that is capable of providing a controlled environment with a uniform distribution of particles with known properties. The aim of this study was to design an exposure chamber for the testing of personal samplers. A polyethylene cylindrical container was used as the testing chamber. Particles generated using a collision nebulizer (BGI) were inserted into the chamber at the top of the chamber. A particle neutralizer was attached to the generation system so as to neutralize the particles before they entered the chamber. Diffusion dryers were used to remove any water vapour from the air stream prior to enter the chamber. Six sampling ports along with a pressure gauge were placed on the walls 15 cm from the bottom of the chamber.

## 18. Workplace measurements of semiconductor nanowires in a small scale producer

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Semiconductor nanowires (NWs) have the potential to be used in applications e.g. solar cells, light-emitting diodes and batteries. Semiconductor NWs are fibre-shaped manufactured nano-objects, and the industrial use is growing as well as the potential for worker exposure. Aerotaxy is a new method for industrial mass production of NWs. Production takes place in closed reactor systems and NWs of GaAs are grown in the gas-phase on catalytic seed nanoparticles of Au by addition of gaseous precursor molecules. The aerotaxy reactor system is opened up during maintenance e.g. cleaning operations. The aim was to quantify the personal breathing zone (PBZ) exposure and emissions of NWs to air during production and laboratory work at a small-scale producer. The potential for dermal exposure was also assessed. PBZ and emission filter samples were collected for 11 production stages for determination of particle number concentration, mass concentration, and metal content. Also direct-reading instruments were used in the PBZ, emission zone and background to measure particle number concentrations and number size distributions with high time-resolution. Tape samples (N=28) were collected from workplace surfaces in the facilities. Preliminary data based on scanning electron microscopy analysis shows concentrations of up to 0.025 NWs/cm3 in PBZ, and 98.4 NWs/cm3 in the emission zone while maintenance was performed. One surface inside the tool enclosure was contaminated with NWs after maintenance. Thus, the results demonstrate workplace exposure to NWs during maintenance, and the personal protective equipment used for this production stage is essential to avoid worker exposure.

## 19. Miniature electrical nanoparticle detector for simultaneous measurement of particle number, average size and lung-deposited surface area

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We present a new, miniature electrical nanoparticle detector, the partector 2. Like its predecessor, the partector, it is based on pulsed unipolar charging, followed by contactless electrical detection of the charges by induced currents in Faraday cages.

The partector 2 contains two Faraday cages for detection, which are separated by an electrostatic precipitator. As in the standard partector, the first Faraday cage electrometer measures an amplitude proportional to the total charge acquired by the aerosol, which is approximately proportional to the lung-deposited surface area of the nanoparticles. In the electrostatic precipitator, small particles are preferentially removed, and thus the electrometer amplitude in the second Faraday cage is reduced. The ratio of the two signal amplitudes on the two Faraday cages is a measure for the average particle diameter of the aerosol. Once the average particle diameter is known, the particle number can be inferred from the signal amplitude on the first cage.

We will present the instrument, first results and also give a comparison to the DiSCmini, an instrument developed previously in our group at the university of applied sciences northwestern Switzerland.

## 21. Multi-parametric surface plasmon resonance living cell sensing for cell interaction studies of nanoparticles

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The surface plasmon resonance (SPR) technique in combination with living cell sensing is a viable platform for novel real-time label-free cell based in vitro tools for pharmaceutical and life science research. Recently, a new configuration of SPR, i.e. the multi-parametric surface plasmon resonance (MP-SPR), has been introduced. The MP-SPR provides the complete SPR spectra in real-time and provides new parameters for analysis compared to conventional SPR. We present here how MP-SPR can be utilized for real-time label-free nanoparticle-cell interaction studies with both animal (e.g. MDCKII) and human (e.g. HeLa, HepG2) cell lines. Examples include proof-of-concept studies showing how it is possible to study targeting efficacy and cell uptake kinetics of nanoparticle based drug delivery systems. Our results collectively show that the MP-SPR based cell assay is a promising new in vitro tool for drug development purposes. In the future, the MP-SPR based cell assay is anticipated to improve the level of information about nanoparticles, such as their potency to cross different cellular barriers, which is not only relevant for treatment of diseases, but also in scope of nanotoxicity. This would enable further standardization of nanomaterials based on in vitro rather than in vivo approach as suggested by the Book of Recommendations for the European Commission by NanoForce. Hence, the MP-SPR based cell assay holds high potential as a novel and powerful assay for nanotoxicity screening and for facilitating the implementation of the Directive 2010/63/EU (i.e. 3Rs policy to replace, reduce and refine animal testing).

## 22. Life cycle, possible release and safety aspects of copper nanoparticles used as additive in cooling agent

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Due to possible health implications after exposure to nanomaterials, the investigation of a possible release along the complete life cycle is becoming increasingly important. This topic was therefore included in the EU funded BUONAPART-E project (Grant no. 280765). The material properties, possible release pathways and exposures to metallic nanoparticles during their life cycle were studied.

The poster will focus on the production of copper nanoparticles and their application as water suspended additive in a cooling agent to improve heat transfer. Results of workplace measurements to quantitatively assess a possible release of airborne particles during production and manual bagging as well as during the mixing with the cooling agent will be presented. Also the release through a sewage plant into surface waters will be assessed.

Furthermore, safety issues related to pyrophoricity of the pure metallic nanoparticles were experimentally evaluated testing for self-ignition and flammability. This piece of hazard information is extended by a literature study on the toxic properties of this type of particles in different compartments. All data are consequently discussed to provide a complete picture of possible threads stemming from the metallic copper nanoparticles along the life cycle.

## 23. Aggregation of aluminum nanoparticles in different electrolytes

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Particles size is considered as the one of the crucial parameter of cytotoxicity of nanosized powders. When solid nanoparticles enter the aqueous medium, they can form suspensions, which can spontaneously aggregate or maintain high aggregation stability. Meanwhile, the aggregation stability of such systems strongly depends on the salinity of the solution. At the same time the addition of Na+ and Ca++ electrolytes is highly necessary to maintain vital functions of aquatic organisms and cells. Our team studies the impact of ionic strength of Na+- and Ca++ electrolytes on the aggregation of Al (90 nm) and Al2O3 (140 nm) nanoparticles in watering suspensions. In this work nanoparticles were dispersed by stirring in previously prepared solution with ionic strength (adjusted with NaCl and CaCl2) of 0.001...100 mM. The concentration of nanoparticles in tested suspensions was 1 mM. The aggregation stability was estimated by the changing of average particles diameter calculated from the particle size distribution measured on the instrument Malvern Zetasizer Nano. According to the experimental data, 48-hour exposure of Al and Al2O3 nanoparticles in aqueous suspensions is accompanied by an intensive aggregation in time: after 48 h, the size of the aggregates increases from 852 nm to 2626 nm for Al nanoparticles and from 618 nm to 1280 nm for Al2O3 nanoparticles. The analysis of the influence of 10 mM electrolytes on the aggregation process at 48-hour exposure has shown that the Al aggregates size of 1953 nm (in water) decreases to 896 nm and 1424 nm in Ca2+ and Na+-solutions, respectively; the Al2O3 aggregates size increases to 2200 nm in both electrolytes.

## 24. Laboratory investigations on the accuracy of personal monitors to measure the individual exposure to nanoparticles

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Personal exposure to nanoparticles can best be assessed by measuring directly in the breathing zone, i.e. within a 30 cm hemisphere around mouth and nose. Nanospecific personal monitors, such as miniDiSC/DiSCmini, Partector and nanoTracer have only become available in the recent years. These instruments are small enough to be carried by a worker without disturbing her/his activities. They are all based on electrical diffusion charging and subsequent charge measurement and determine the airborne particle concentrations with high time resolution. While the typical comparability of these monitors has been shown to be around ±30%, the accuracy of the instruments as a function of particle size has not been investigated in the past. In the present study we investigated the accuracy of the monitors by generating monodisperse particles of various sizes and comparing the results of the monitors with reference values. The monodisperse particle sizes ranged from 10 nm to 700 nm and were chosen to be both within and beyond the specified size ranges of the monitors. Furthermore both spherical and agglomerated particles were used. The investigations revealed a fairly strong size dependence of the instruments' accuracy. It is typically well within the aforementioned ±30% range as long as the particle sizes are between 20 and 300 nm. Deviations for particle sizes outside of this range can be significantly larger.

## 25. Release of nanoparticles from dental composites during restoration grinding and polishing

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Modern dental composite materials used for tooth restoration are made from a combination of polymer resin with e.g. SiO2 or ZrO2 filler particles. The filler particle contents are high, usually >50 vol%. While classic dental composites contained filler particles with sizes of several hundred nanometers up to micrometers, current materials typically also contain filler particles below 50 nm, which may be released during different treatment steps, such as restoration grinding or polishing, which in turn may cause uptake via inhalation by both the dentist and the patient. We here present a thorough study which involved both the measurement of dentists' personal exposure as well as laboratory studies under defined conditions to characterize the particles released from a dental composite during grinding.

To characterize dentists' personal exposure, the number concentration, lung deposited surface area concentration and mean particle size were measured in the dentist's breathing zone with a miniDiSC. Simultaneously, a second miniDiSC instrument was placed approximately 5 m away to monitor particle background. The results show that very high concentrations of sometimes >1e6 1/cm$^3$ are released during the various mechanical treatment steps of dental composites.

Different composite materials were subject to intensive release investigations under defined laboratory conditions. The submicron number size distributions of the airborne particles were measured and particles sampled for consecutive analyses. The investigations revealed that in all cases very high concentrations of particles mostly smaller than 100 nm were released, which agrees well with the personal exposure measurements. In some cases, single engineered nanoparticles were detected.

## 26. Surface-reactivity of metal and metal oxide nanoparticles in aqueous solutions

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Metal and metal oxide nanoparticles are known to make a cause for anxiety due to their potential impact on the environment and humans. Biological tests of nanomaterials require preparation of nanoparticles suspensions having stable dispersion and elektrokinetic parameters in watering medium. High stability of suspensions is mostly achieved when using chemical stabilizers which adsorption is associated with acid-base state of nanoparticles surface. Our research was focused on the study of surface-reactivity of Zn, ZnO, Al, and Al2O3 nanoparticles in aqueous solutions of different acidity. The active acid-base centers on nanoparticles surface were determined by means of visual colour change method using the Hammett indicators. The obtained results have shown the presence of neutral (Zn---OHδ°), basic (Zn---OHδ-) and acid (Zn---OHδ+) Bronsted centers on Zn and ZnO nanoparticles surface. Metal Zn nanoparticles more often had stripes that corresponded to acid centers (pKa < 6.4), whereas ZnO nanoparticles spectrum had wide stripes in the base part of pH scale (pKa >7.3). Acid-base properties of Al nanoparticles were characterized by acid (pKa= 5.5; 6.4) and basic (pKa= 8.0; 9.4; 12.8) Bronsted centers indicating weakly acidic surface state. The spectrum of active centers on the surface of Al2O3 nanoparticles was mostly pronounced by following stripes: pKa = -0.29; 1.1; 4.9; 9.45; 13.3. A wide range of active centers indicate the amphoteric state of the surface of Al2O3. Generated findings allowed revealing the degree of solution acidity where nanoparticles are likely to have the highest adsorption activity.

## 27. Comparison of particles concentrations obtained with portable and laboratory devices

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The exposure to nano-objects can be characterized by number and surface concentrations and size distribution of nano-size particles. For measurements of those parameters, also in real conditions, usually are used laboratory devices with large dimensions.

This work presents the results of comparisons of number and surface concentrations obtained in real conditions with laboratory (SMPS, P-Trak, CPC 3775, CPC 3007, Aero-Trak) and portable (DiscMini's) devices, before, during and after processes of production of TiO2 powders and mixing powders of three types of nano-objects: nanoparticles (SiO2 powder from Sigma-Aldrich), nanoplates (nanocly-nanomer I.34MN) and nanotubes (multi-wall nanotubes MWCNTs 10-20).

Data shown good correlations between results obtained with portable DiscMini's and devices with large dimensions, excluded some very high maximum values obtained with DiscMini's during processes of mixing nanomaterials powders. The reasons of differences can be very many, e.g.:
- very short time of mixing (about 30 s each),
- different size range of particles measured with used devices: SMPS (16-661 nm), P-Trak (20-1000nm), Aero-Trak (10-1000nm), DiscMini (10-700nm),
- different time of devices response (from 1 s to 3 min.),
- possible different direction of air flow close to the sampling points,
- different delivery of air sampling to the devices: to SMPS, P-Trak and Aero-Trak through the tubes and to DiscMini's directly by separator, without the tubes.

When maximum values were not very high good correlations for those values were also observed.

This work presents the results of the projects: SCAFFOLD (GA 280535) and 2.Z.04 (CIOP-PIB).

## 28. Lessons learned from measurements of incidental nanoparticles at workplaces in the Czech Republic

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Occupational exposures to airborne particles present a long-term concern for industrial hygienists. The ability of smaller particles to get deeper into human respiratory system is connected with serious health effects. Nowadays, the issue is of high priority since materials in nano size are intentionally manufactured for their unique properties. With the rapid development of these so called engineered nanomaterials (ENMs) and the need to ensure their safety, great progress in the instrumentation for exposure measurements and ENMs characterization has been made. The instrumentation can help to better understand exposures to incidental nanoparticles as well.

Incidental nanoparticles are of high interest in the Moravian-Silesian Region, Czech Republic, which is a hub of iron and steel industry. The Institute of Public Health Ostrava and the VSB-Technical University of Ostrava conducted preliminary surveys on airborne particulates at different workplaces to access occupational exposure to nanoparticles in the light of advanced methods for their monitoring and characterization. Collected information on mass and number concentration, particle size distribution, surface area of alveolar and tracheobronchial deposition fractions, SEM-EDAX characterization and ICP-MS analysis provided valuable input for chemical safety management. However, the results should be interpreted with caution. Spatial variability in both particle size distribution and number concentration and evaluation of background concentrations present the main challenges. Routine hygiene measurements of nanoparticles (incidental as well as engineered) in working environments would require well trained staff involved in an international network of knowledge exchange and best practices sharing.

## 29. Analysis of nanoparticle-containing sunscreens with a miniaturized Asymmetrical Flow Field-Flow Fractionation (mAF4) cartridge

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Despite the many advantages of Asymmetric Flow Field-Flow Fractionation (AF4), its adoption for routine use is limited by the large size of currently available separation cartridges. The goal of this project was a miniaturization of the AF4 cartridge and its application to the analysis of complex nanoparticle-containing samples such as commercial sunscreens. The advantages of a cartridge scale-down include simplified handling, reduced costs and higher throughput capacities.

A miniaturized AF4 cartridge was fabricated with a channel design similar to that used in larger FFF cartridges. By adapting the measurement protocols (application time and intensity of the various flows) to the new channel geometry, separation efficiencies comparable to conventional AF4 cartridges could be maintained, despite a channel length of less than 7 cm.

Initially, the setup was applied for the separation of a range of gold and silver nanoparticle mixtures (5 – 80 nm). After the initial characterization, we applied our platform to the analysis of commercial sunscreens containing TiO2 nanoparticles. The analysis of nanoparticles in complex matrices and consumer products is a pressing problem that has to be faced by manufacturers, mainly due to newly enforced regulatory requirements (e.g. European Cosmetics Regulation). Those new requirements clearly call for cost-effective measurement systems to be operated in routine laboratories (e.g. QC lab of cos-

metic production facility). In this context, the miniaturized AF4 cartridge could play a crucial role and help to promote FFF technologies in the everyday lab as a valued tool for the analysis of nanoparticle-containing products.

## 30. Occupational contact with the nanoparticles: strategy of detection and analysis

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Currently, rapid development of high-tech using nanoparticles in the various branches of science and human activities observed in technology, biology, medicine. This is due to the fact that nanotechnology will provide a unique opportunity to carry out the manipulations at the level of one or few nanometers, which effectively determines the management of physical, chemical and biological processes at the atomic and molecular levels. Development of nanotechnology actually happens against the background of the lack of practical knowledge about the impact of nanoparticles on human health (possible mechanisms of the interaction of nanoparticles with biological objects). Development of appropriate approaches to the prediction of risk of nanoparticles on human health and inseparably linked with the study of the fundamental laws of it biological effects. Either, is not established the possible adverse effects of nanoparticles on the organism working in professional contact with the nanoparticles.

Risk factors of occupational and work-related respiratory diseases are works involving exposure to chemicals, also dusts (include nanoparticles).

The mucosal epithelium lining the upper and lower conducting airways provides a barrier against injury from inhaled toxicants, dusts, and biological agents. The normal respiratory epithelium is coated with mucus which lubricates, insulates, and humidifies the epithelium and protects it by entrapping of these agents. This study will examine epithelium cell uptake (are released from nasal lavage) of various nanoparticles in working places: metallurgical, wood productions and office (control). In order to investigate similarities and differences in mucous secretion in response to inhaled toxicants by scanning electron microscopy.

## 31. Comparison of nanosized particles and gaseous compounds in different work environments

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Indoor air pollution in occupational environments can significantly affect worker´s health factors. Studies suggest that exposure to nanosized particles may increase pulmonary inflammation, effects on circulation and even death [1]. Knowledge of types of indoor air pollutants and concentrations, as well as exposure time, is needed to estimate the health effects.

Here, four different workplaces were studied in order to determine the levels of nanoparticles and gaseous compounds in air. Workplaces from industry, service, handicraft and research sectors were selected for the study from which one workplace utilized engineered nanomaterials (ENM) in their line of production. Particle number concentrations and size distributions were measured continuously from few days to a week depending on studied process and work environment. The concentration of volatile organic compounds (VOCs) were measured in order to study the relationship between gas and particle phases.

Transmission electron microscopy (TEM) was used to further characterize particles.

We compared different workplaces and their levels of particles and gaseous impurities. Based on the measurement results, we recognized work processes and work phases that influenced the concentrations of nanoparticles and gaseous compounds. Some indoor sources identified for nanosized particles included: smelting plastic, polyurethane molding and spraying. Some particle sources were located to outdoors.

Reference:
1. Savolainen, K., et al., Nanotechnologies, engineered nanomaterials and occupational health and safety – A review. Safety Science, 2010. 48(8): p. 957-963.

The Finnish Work Environmental Fund (no.112132 and no.112133) is acknowledged for funding.

## THEME : RISK ASSESSMENT AND CONTROL

### 33. Practical application of the Scaffold - Toolkit for the management of nano-risks in construction.

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Construction in the European Union is a dynamic sector and the biggest industrial employer (3,1 million enterprises - 95% have less than 20 workers - and 14,9 million jobs). The increasing use of MNMs and nano-enabled products in construction might pose new health and safety risks to workers at different stages of the life cycle in construction. Consequently companies need to address the management of these potential occupational emerging risks.

SCAFFOLD (GA 280535) is an industrial oriented project, specifically focused in providing practical solutions for construction, regarding current uncertainties about occupational exposure to MNMs. The project has developed an innovative Toolkit to support the management of risks derived from MNMs, based on consistent state of the art OHS management models (OHSAS 18001 + ISO 31000, crossing contents with the future ISO 45001).

This paper presents the application of the Scaffold -Toolkit in five Industrial Case Studies (ICS) representative of the sector. These practical experiences involve several kinds of MNMs and nano-enabled products, and cover three stages of the life cycle of MNMs in construction: manufacturing of nanomaterials and nano-enabled products, their uses in construction sites and finally, disposal in demolition field. The companies involved in ICS are located in three European countries: Poland, Rumania and Spain. This work will provide data on risk management jointly with specific issues about management of MNMs as arisen from experience.

## 35. Evaluation principles of nanoparticles in dusty occupational environments

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Despite numerous possibilities for measurement and assessment of various particles (including total, inhalable and respirable dust particles) at workplaces there is still no common well established and tested approach for measurement of nanoparticles and their exposure assessment especially in occupational environments. There is lack of established measurement standards as well as low-cost affordable equipment and instrumentation. Most of current research is targeted towards scientific purposes using such nanoparticles as TiO2, ZnO and carbon nanotubes intended to characterize nanoparticles and test their toxicity. However there are very few studies on practical evaluation of nanoparticles and their health effects in practical occupational environment. Current research efforts are also concentrating on parameters and better detection methods for assessment of potential risks of nanoparticles with some results available already. Still as there is a lack of useful data on real exposure in various industries and there for a project "The development of up-to-date diagnostic and research methods for the risks caused by nanoparticles and ergonomic factors at workplaces", Agreement No. 2013/0050/1DP/1.1.1.2.0/13/APIA/VIAA/025 has been started with the aim to develop and test new methods for nanoparticle identification, characterization and exposure assessment in metal (for metal fumes and paints) and woodworking (for wood dusts in grinding processes) industries. Various methods of measurements (e.g. ELPI+ ) will be tested in workplaces for their practical application and for quantification and assessment of nanoparticles (including full chemical analysis and electron microscopy). For evaluation of health effects we plan to use analysis of nasal lavage fluids. First analysis has started and we expect to have results by early 2015.

## 36. The applicability of Stoffenmanager Nanotool in risk assessment of nanomaterials and comparison to the airborne particle measurements at selected workplaces

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Control banding tools have been developed for risk assessment of handling engineered nanomaterials (ENM) at the workplaces, because of gaps in the hazard and exposure data of ENM.

The applicability of the control banding (CB)-approach, more specifically the freely available control banding tool Stoffenmanager Nano 1.0, in assessing and managing risks of nanomaterials was studied. The results of the selected CB-approach were compared with the expert judgement, focusing mainly to the exposure rather than hazard, since the expert evaluation was based on contextual information of the products and tasks, visual observations and airborne particle measurements at the workplace, with no OELs available for the used nanomaterials.

The risk and exposure assessment by using the CB-approach and the expert evaluation was carried out in seven companies and in two pilot cases. The tasks performed by the companies related to application of a coating (floors, walls, HVAC products etc.), handling nanopowder or CNT, and manufacturing paints. Two pilot cases included spraying of sol-gel coating with nano-TiO2 and manufacturing de-pollutant mortar with nano-TiO2.

The presentation will compare and discuss the exposure levels acquired from the CB-tool to the airborne particle measurements. The results indicate that the Stoffenmanager Nano tool is applicable in some of the studied industrial workplaces, and the selection of the source domain is clear and easy. However, there could be more detailed input information, i.e. explanations and examples on the handling process e.g. example descriptions of work, and amounts of used product, in the task and activities domain.

Studies were performed in the EU FP7 projects Nanodevice (GA CP-IP 211464-2), MARINA (GA 263215) and Scaffold (GA 280535), and The Finnish Work Environmental Fund (no.112132 and no.112133.

## 37. Efficiency evaluation of disposable coverall against nanoparticles in conditions simulating occupational use

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Despite their potential toxicity, use of nanoparticles (NP) is a growing concern in the workplace. While the most direct pathway for NP exposure in the workplace is inhalation, the dermal contact pathway is also a concern. Indeed, some studies indicate that NP can penetrate through the different dermal layers. Accordingly to protect workers against dermal exposure, the precautionary approach to risk management recommends the use of protective clothing against chemical agents. However, very few scientific studies are available to prove their effectiveness against NP penetration. Moreover, the current methods used are based exclusively on the penetration of particles without taking into account the constraints as experienced by the garments under actual conditions of use.

To address this problem, a test setup and a sampling protocol have been developed for measuring the penetration of nanoaerosol through the materials of disposable coverall (DC) under conditions simulating their use in workplace.

This study compares the performances of four different DC commonly used in industry against submicrometer particles. DC material samples are exposed to a controlled sodium chloride aerosol produced by nebulization. The sample is placed in a test chamber wherein the aerosol is generated upstream of the material. During the particles exposition, the sample is subjected to mechanical deformations simulating occupational use. The size distribution of the aerosol downstream is determined in real time by an engine exhaust particle sizer (EEPS).

The results obtained help to predict the penetration of nanoparticles through DC and some recommendations are made to enhance the safety of workers.

## 38. How to store nanomaterial safety data: meet eNanoMapper database

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Nanomaterial safety assessment has become an important task following the production growth of engineered nanomaterials (ENMs) and the increased interest for ENMs from various academic, industry and regulatory parties. A number of challenges exist in nanomaterials data representation and integration mainly due to the data complexity and origination of ENM information from diverse sources. We have recently described eNanoMapper database (http://nanoinfo2014.weebly.com/program.html) as part of the computational infrastructure for toxicological data management of engineered materials, developed within eNanoMapper project (http://www.enanomapper.net). The eNanoMapper approach builds on previous experience of the consortium partners in supporting diverse chemical data through flexible data storage, semantic web technologies, open source components and web services. The shift from the chemical structure based approach to a substance based information management enables support for the new challenging cases of ENMs.

The supported import formats (OECD HT, custom RDF and CSV formats) are currently being extended with ISA-TAB-Nano and a large set of custom spreadsheet templates, taking into account the observation that the latter is the preferred approach data preparation format of the majority of the NanoSafety Cluster projects. A configurable parser enables import of the data stored in the supported set of spreadsheet templates, accommodating different row-based, column-based or mixed organization of the data. The configuration metadata is defined in a separate file, mapping the custom spreadsheet organization into the internal eNanoMapper storage components: Substance, Protocol, Measurement, Parameters and Conditions. This enables uniform approach towards import, storage and searching of the ENM physicochemical measurements and biological assay results.

## 39. Recommendations for occupational health care units concerning workers handling nanomaterials in the construction sector

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Nanomaterials are increasingly being used in construction products. Workers' exposure to nanomaterials may mainly occur during activities generating dust or in applications involving spraying. Also dermal exposure may occur.

In the Scaffold project (GA 280535), a guidance document for occupational health care units concerning exposure to nanomaterials at the construction sites was developed. The first step involves the identification and listing of the products used at the workplace, including identification of products containing nanomaterials. Next, the potential exposure sources should be identified and a risk assessment conducted using, for example, Control Banding (CB) methods or the help of experts. In the risk assessment it is important to consider not only nanomaterials, but all kinds of agents, including traditional chemicals and dusts. In many cases traditional chemicals, e.g., solvents are likely to pose a much higher health risk than the nanomaterials. Based on the outcome of the risk assessment, the required engineering controls and other essential risk management measures have to be implemented. For workers using nanomaterials, the establishment of a nanoexposure registry is recommended. It should contain information on nanomaterial-containing products, the likely exposed workers, activities and tasks, and the estimated level of exposure. Using data from the registry, exposure-disease (health effect) associations may be determined in the future. The established medical surveillance approaches for construction workers with periodic health examination every 1 to 5 years can be applied as such. For workers regularly exposed to nanomaterials, a follow-up, focusing on the respiratory system is recommended.

## 40. iNanotool - Development of an interactive tool for the implementation of environmental legislation into nanomaterial manufacturers' operations

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Companies involved in nanotechnology, mainly SMEs, have in general a high technological component. Wast number of companies are spin-offs, that have emerged from existing research institutions (mostly universities). According to different studies high percentage of the nanomaterials producers in Europe are micro and small enterprises.

Nanomaterials production involves several potential environmental risks directly related to the production process (production, functionalisation and incorporation into nanocomposites). Nanowastes generated could comprise items such as contaminated wipes, filters and discarded components. Assessment of their impact is a function of their intrinsic characteristics, the manufacturing process, size of batch production, storage and distribution.

The objective of i-NanoTool project is to contribute to the efficient implementation of the environmental policy and legislation into the processes of companies manufacturing nanomaterials, especially SMEs. During the project an interactive platform (etool) for environmental self-diagnosis addressed to nanoparticles manufacturers in European countries is developed.

i-NANOTool project's etool is tested with the help of some nanotechnology companies from Spain, Portugal, Finland and Romania. This etool will give information about the current environmental legislation and methods to evaluate the environmental impact. It will analyze the grade of compliance of the companies in relation to the legislation and provide recommendations to improve their situation according to the self-diagnosis. Etool provides guidelines for managing the environmental impact, both during production process and in the end of the life-cycle. i-NanoTool project is funded by LIFE+2012 Program (LIFE12 ENV/ES/000326).

### 41. In vitro cellular stress response to routinely used paint particles, and assessment of paint factory particle aerosol generation

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The assessment of workplace exposure is an important issue within all industries, ensuring safe working conditions and limiting occupational exposure. We have assessed the in vitro response of human lung alveolar cells to powder components collected in a paint production factory; including dolomite, kaolin, talc, three silica and four TiO2 samples.

Aerosols generated during powder handling activities were characterised by FMPS, ELPI and CPC, surface area by BET, and suspended particle size by DLS and TEM. The dustiness test was used to determine high particle release, indicative of an accident scenario. A549 cells were exposed to 10–500µg/ml and assayed for cytotoxicity and IL-8 secretion. Two silica, one TiO2 and kaolin were further assessed using A549 RFP reporter cells under the control of IL-8, NF-κB and HO-1, as these had induced cytotoxicity and IL-8 secretion in our first assays and all other materials induced no adverse effect. All four particles induced dose- and time-dependent increase in IL-8, NF-κB and HO-1, indicating IL-8 secretion was related to oxidative stress and activation of the transcription factor NF-κB.

These data indicate that common components of mass-produced paint can induce both pro-inflammatory conditions and affect the REDOX activity of lung cells. The concentrations used here were relatively high, and therefore the relationship between lung deposition and daily occupational exposure, or accidental high release, will be assessed with the use of mathematical modelling.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement No: 263147.

## THEME : ENVIRONMENT: SOURCES, FATE AND CONTROL

### 43. A comparison of silver nanoparticles and silver ions behaviour at anaerobic conditions and effect on biogas production

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Silver nanoparticles (AgNPs) are currently one of the most manufactured nanomaterials. The release of AgNPs as well as silver ions from industry and consumer products has been reported which result in reaching these to wastewater treatment plants.

To compare the effects of these two forms of silver on anaerobic digestion the batch tests according to ISO 13641-2 method were performed investigating the effect on biogas and methane production. The experiment was carried out with collargol (CAS No. 9007-35-6) and silver nitrate (CAS No. 7761-88-8).

The main results showed that spiking with silver soluble salt had negative impact on biogas production at the concentrations higher than 80 mg Ag/L. The studied AgNPs did not show any significant effect on biogas production up to concentration of

320 mg Ag/L. At the beginning of experiment at higher concentrations of AgNPs the methane production was slightly inhibited, however after several weeks there were no significant effect of AgNPs on methane production either. The effect of the AgNPs surface capping agent in anaerobic digestion, providing an extra substrate, could conceal the possible negative effect of silver in the experiments.

To study the shedding of Ag ions from AgNPs and dissolution of silver in our test conditions, the concentrations of soluble silver in test medium was measured with atomic absorption spectrometry. The concentrations of shedded Ag ions in different tests and relevance of the dissolved ions in inhibition of biogas and methane production will be presented.

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## 44. Addressing the complexity of water chemistry in environmental fate modeling for engineered nanoparticles

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Environmental fate models for engineered nanoparticles (ENPs) still have limited applicability because they employ constant environmental conditions along the modeled system or system-specific environmental representations. Both approaches do not provide a general understanding of the effects of spatial and/or temporal variability. To address this gap, we developed a novel modeling strategy that: 1) incorporates spatial variability in environmental conditions in an ENP fate model; and 2) analyzes the effect of a wide range of randomly sampled environmental conditions. Using this approach, we investigated the transport of nano-TiO2 in the Lower Rhone River (France) under numerous scenarios of environmental conditions. The spatial concentration profiles of nano-TiO2 predicted for all of these scenarios were then grouped according to their similarity by using cluster analysis. The analysis resulted in a small number of clusters representing different groups of spatial concentration profiles. Analysis of the features of each cluster demonstrated a strong association between the water conditions in regions close to the ENPs emission source and the cluster membership of the corresponding spatial concentration profiles. In particular, water compositions favoring heteroaggregation between the ENPs with suspended particulate matter resulted in clusters of low variability. These conditions are, therefore, reliable predictors of the eventual fate of the modeled ENPs. Our results, indicate that the focus of future modeling and experimental research of ENP environmental fate should be on the water characteristic in regions near the expected ENP emission sources. Under conditions favoring heteroaggregation in these regions, the fate of the ENPs can be readily predicted.

## 45. Environmental remediation of oxidised graphene nanocarbons: 2D sheets degrade faster than 1D tubular-shaped structures

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Graphene nanocarbons are currently fuelling a revolution in science and technology in areas ranging from aerospace engineering to electronics. Unlike their pristine forms, the oxidised derivatives of those nanostructures are water dispersible that allows their application to areas such as biology and medicine. There is a need for efficient and viable means of environmental remediation of these engineered structures. The aim of the present study was to assess the potential of the widely used NaClO (1% by chlorine content) to degrade oxidised graphene nanocarbons within a week, for instance in wastewater treatment. We compared the morphological changes that occur during degradation of graphene oxide to two other oxidised graphene nano-

carbons, namely oxidised multiwalled carbon nanotubes and oxidised nanohorns. Degradation was monitored closely using a battery of techniques including visual observation, UV-Vis, Raman spectroscopy, transmission electron microscopy and atomic force microscopy. The results demonstrate that graphene oxide is degraded into a dominantly amorphous structure lacking the characteristic Raman signature and microscopic (TEM/AFM) morphology. Oxidised carbon nanotubes underwent degradation via a wall exfoliation mechanism yet maintained a large fraction of the sp2 carbon backbone, while the degradation rate of oxidised carbon nanohorns was observed at a somewhat intermediate rate to that for the other two types of nanostructures.

## 46. Evaluation of manufactured nanoparticle heteroaggregation with suspended particulate and dissolved organic matter in natural surface waters

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In predicting the fate of engineered nanoparticles (ENPs) in natural surface waters, environmentally relevant conditions should be applied with regard to system composition and the ENP concentration (µg/L range). These conditions are likely to favour heteroaggregation of the ENPs with naturally occurring suspended particulate and dissolved organic matter. Herein, we evaluated the fate of titanium dioxide (TiO2) nanoparticles in surface waters sampled from a river (Rhône river, France) rich in mineral suspended particulate matter (SPM) and a lake (Cholet, France) with high natural organic matter (NOM) content. The TiO2 nanoparticles were spiked into each of these systems, and the kinetics of heteroaggregation and the sticking efficiencies between the nanoparticles and the natural suspended matter were determined. Studies were also conducted in synthetic waters of comparable composition to better understand the driving physico-chemical factors in the observed heteroaggregation. Furthermore, the pH, ionic strength, elemental composition, and SPM and NOM contents of the waters were assessed to elucidate the key contributors in NP fate. Under suitable physico-chemical conditions, the TiO2 nanoparticles demonstrated a significant affinity for the mineral SPM, with rapid heteroaggregation of the system and sedimentation of the resulting aggregates. Heteroaggregation was less evident in the NOM rich lake water at environmentally relevant ENP concentrations. Together, these holistic data, coupled to a river-scale fate model, will aide in ranking potential ENP fate scenarios and assessing ENP risk within natural aqueous environments. Work funded by the French National Research Agency and the Swiss FOPH as NANOHETER under the frame of SIINN. http://nanoheter.cerege.fr

## 48. Development of a surface based sensing tool for characterization of nanoparticle functionalization and corona formation

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Nanoparticles introduced in a biological environment are inevitably coated with a diversity of molecules, e.g. proteins, sugars and lipids. This coating (corona) largely determines the biological activity of the nanoparticle and it is therefore urgent to characterize, understand and control these adsorption processes.

For this purpose, we have developed a new version of indirect nanoplasmonic sensor surfaces that utilizes spheroidal Au-nanoparticles grown on a flat support and covered by 10 nm of SiO2 to form a core-shell structure. As we show, these nanostructures can be considered as surface-bound mimics of SiO2 nanoparticles in suspension with built-in sensing function. Upon illumination with white light the plasmon resonance in the Au core is excited. Adsorption of molecules to the SiO2 shell will spectrally shift the plasmon resonance, which manifests itself as a shift of the “peak” in the extinction spectrum. In this manner, adsorption events on the curved nanoparticle surface can be detected in situ and in real time.

To demonstrate this concept, we have fabricated sensor surfaces with SiO2 nanoparticle mimics of different curvature and show their high-accuracy response to changes in their local environment. In addition, we applied silanization treatment on the sensor nanoparticles to alter protein adsorption behaviour.

The versatility of the described technology, through the possibility to control the nanoparticle curvature and surface chemistry, enables analysis of sets of well-defined surfaces. Thus, we believe that this sensing methodology will prove itself useful in the fields of nanotoxicology and nanomedicine, when characterizing surface transformations of nanoparticles.

# POSTER SESSION 2
# Tuesday 14 April 2015

## THEME : NANOTOXICOLOGY

### 51. Systemic toxicity and genotoxicity of copper oxide nanoparticles as assessed in a subchronic experiment on rats

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In the copper metallurgy workplace air is polluted with condensation aerosols which a significant fraction of is presented by CuO nanoparticles. In the scientific literature, there is a lot of experimental evidence of such nanoparticles' high cytotoxicity in vitro but their in vivo toxicity characterization is not sufficiently substantiated.

A stable suspension of CuO particles with mean diameter 20nm was prepared by laser ablation of pure copper in de-ionized water. This suspension was injected intraperitoneally to rats at a dose of 10 mg/kg times 6 weeks up to 19 injections. In parallel, another group of rats was so injected with the same suspension against the background of oral administration of a "bio-protective complex" (BPC) comprising pectin, a multivitamin-multimineral preparation, some amino acids and fish oil rich in omega-PUFA. After termination of injections, many functional and biochemical indices for the organism's status as well as pathological changes of liver, spleen, kidneys and brain microscopic structure were evaluated. In the same organs we have measured accumulation of copper while their cells were isolated and used for performing the RAPD test for DNA fragmentation. The same features were assessed in control rats infected intraperitoneally with water with or without administration of the BPC.

The investigated CuO nanoparticles proved adversely bio-active in all above-mentioned respects. Some features of the induced subchronic intoxication may be considered specific for copper toxicity.

The BPC administration diminished copper retention in tissues and attenuated both toxicity and genotoxicity effects of exposure to these nanoparticles.

## 52. Effect of aerial exposure of silver nanoparticles on photosynthetic performance of broad beans (Vicia faba)

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The incorporation of nanoparticles into consumer products has significantly increased over recent years. Many of these products contain silver nanoparticles (Ag NPs) due to their well-known antimicrobial properties. Despite these and other industrial advantages, concerns have been raised as to the potential environmental effects that may occur by the release of Ag NPs from such products. However, to date there is currently very little information available regarding the effects of Ag NPs on plants.

In this study, the effects of methoxy-polyethylene glycol capped silver nanoparticles (cAg NPs) on the photosynthetic performance of broad beans (Vicia faba L.) were investigated. The results showed clearly that when cAg NPs were injected into the leaves at concentrations of 50 and 100 mg/L significant reductions in 1) both the maximum quantum efficiency of PSII photochemistry (Fv/Fm) and the operating efficiency of PSII photochemistry (Fq'/Fm'); 2) carbon assimilation rate (A); and 3) stomatal conductance (gs) were apparent. Further investigation showed that this was not due to the production of damaging reaction oxygen species and that decreasing photosynthesis was possibly due to the accumulation of cAg NPs inside the internal leaf spaces.

This study strongly demonstrated that cAg NPs have the potential to affect several plant processes that will potentially impact on plant productivity. Such effect could diminish the food production demand that is urgently needed to meet the growing increase in population. Therefore, investigations as to the effects of Ag NPs and the potential consequence of them reaching the food chain are of paramount importance.

## 53. Comparison of different in vitro models of the blood-brain barrier for study of toxic effects of engineered nanoparticles

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Due to their new physico-chemical properties engineered nanoparticles (ENPs) are increasingly employed in numerous industrial sectors (such as electronics, textile, aerospace, cosmetics, pharmaceuticals, food industry, etc). These new physico-chemical properties can also represent a threat for the human health. Consumers can notably be exposed involuntarily by different routes such as inhalation, ingestion or through the skin. Several studies recently reported a possible biodistribution of these ENPs on the blood-brain barrier (BBB). Consequently, there is a great need for developing BBB in vitro models representative of the in vivo situation and capable of rapidly and accurately assessing ENPs toxic effects and their potential translocation through this barrier.

In this study, several in vitro models established with micro-endothelial brain cell lines of different origins (bEnd.3 mouse cell line or a new human cell line) co-cultivated or not with astrocytic cells (C6 rat or C8-B4 mouse cell lines) on Transwells® were compared using different endpoints: trans-endothelial resistance, permeability of the Lucifer yellow and protein junction labeling. Impact of NIST diesel exhaust particles on BBB cell viability is also discussed.

## 54. Proteomics of CNT-exposed cells reveals cell-type-specific and carbon nanotube subtype-specific systemic effects

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Introduction: Toxicoproteomics is a developing field to investigate the physiological response as a result of adverse toxicant exposure. The aim of this study was to compare the proteome-wide mechanistic differences following exposure to two different kinds of short carbon nanotubes (CNTs).

Methods: We used three different cell types to mimic the exposure route to CNT aerosols; a human lung fibroblast (MRC9) and a human lung epithelial (A549) cell line and monocyte-derived human macrophages, the first line of defense against airway exposure. Cell monolayers were exposed (31.5µg/cm2) to either multi-walled or single-walled CNT for 24h. Unexposed cells were taken along as controls. Label-free proteomic characterization of enriched cytosolic fractions was carried out according to standard procedures using an nLC-MS/MS platform (QExactive). Identification/quantification was done with MaxQuant followed by differential abundance and pathway enrichment analysis using Perseus.

Results: A total of 4280 unique proteins were identified in the A549 cells, 2845 in the MRC9 cells and 3800 in the monocyte-derived macrophages. Multivariate analysis of differentially abundant proteins successfully clustered all cell types and corresponding biological replicates into treatment groups consisting of unexposed cells and cells exposed to either mwCNT or swCNT.

In total 176 protein groups were differentially abundant (Benjamini Hochberg FDR of 1%) between treatments in A549, 199 protein groups in MRC9 and 469 in macrophages. Both cell- and CNT-type specific proteins were observed. Enriched canonical pathways involved inflammation, proliferation, oxidative stress and development of cancer.

A proteomic profiling comparison between cell-based models and in-vivo CNT exposure emphasized a true biological response and provides a framework for mechanistically assessing the cellular reactions to engineered nanomaterials.

## 55. Benchmark dose analysis (BMD) of CuO and WCCo nanomaterials cytotoxicity in macrophage versus hepatocyte cell lines

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This study performed within the frame of FP7-SUN (Sustainable Nanotechnologies) aimed to evaluate the effects of nanomaterials (NMs) in two different cellular models, macrophages and hepatocytes, respectively. The NMs selected for this study were the pristine forms of copper oxide (CuO) and fine tungsten carbide with cobalt binder (WCCo). Additionally the cells were exposed also to $CuCl_2$ and $CoCl_2$. The cytotoxicity and the inflammatory potency of NMs were investigated in RAW264.7 macrophages and C3A hepatocellular carcinoma cell line after 24h of exposure. The cell viability was measured using the Alamar Blue (resazurin) assay and the results were used for benchmark dose (BMD) analysis by PROAST 38.9 software. The $BMD_{20}$ and $EC_{50}$ parameters were used to compare the results between the cell lines and the NMs. The results in RAW264.7 cells showed that CuO NMs ($BMD_{20}$=25.50 µg/ml; $EC_{50}$=40.97 µg/ml) were more toxic than WCCo NMs ($BMD_{20}$=48.10 µg/ml; $EC_{50}$=98.08 µg/ml). Also in C3A cells, CuO NMs showed a higher toxicity ($BMD_{20}$=25.80 µg/ml and $EC_{50}$=32.54 µg/ml) compared to WCCo NMs ($BMD_{20}$=157 µg/ml and $EC_{50}$>200 µg/ml). Further studies will include the evaluation of the released and aged forms of the same NMs and also the single components of the NMs (i.e. WC vs. Co). This will allow us to understand the possible adverse effects of NMs along their entire life cycle.

**Keywords**: copper oxide, tungsten carbide, nanomaterials, in vitro toxicity, benchmark dose

## 56. Single-wall or double-wall carbon nanotubes induce atherosclerosis progression in animal and culture models of atherosclerosis

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Background: Recently, the use of carbon nanotubes has been increasing rapidly in a variety of fields. However, cardiovascular effect of exposure to carbon nanotubes remains elusive. The present study investigated the effects of pulmonary exposure to single-wall carbon nanotubes (SWCNT) or double-wall carbon nanotubes (DWCNT) on atherosclerosis progression in normal human aortic endothelial cells (HAECs) and apolipoprotein E (ApoE) null mice.

Methods and results: ApoE null mice were exposed by pharyngeal aspiration to SWCNT or DWCNT (10 or 40 μg/mouse) once every other week for 10 weeks. Oil red O staining showed increase in the plaque areas of the aorta in ApoE null mice exposed to SWCNT or DWCNT compared with the vehicle-treated ApoE null mice. The expression of the adhesion molecule (ICAM-1) was increased on the aorta in ApoE null mice exposed to SWCNT or DWCNT. We isolated the endothelial progenitor cells (EPCs) from bone marrow of exposed mice and analyzed their ex vivo function. Seven days after the end of exposure, colony-forming units and migration assays were performed. Exposure to SWCNT or DWCNT at high dose reduced colony-forming units. Moreover, exposure to SWCNT at high dose decreased the number of migration cells. HAECs were cultured and exposed to carbon nanotubes. High-dose SWCNT or DWCNT reduced cell viability, increased the expression of ICAM-1, and enhanced adhesion of the THP-1 monocyte to HAECs.

Conclusion: The study suggested that SWCNT and DWCNT induced atherosclerosis progression through reduction of the function of EPCs and enhancement of adhesion of monocytes to the endothelial cells.

## 57. Characterization of mesothelioma induction by i.p injection of the MWCNT dispersed with the Taquann method

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We had previously reported that dose-dependent mesothelioma induction by the i.p. injections of MWCNT in p53+/- mice. Peritoneal fibrosis, peritoneal adhesion and formation of foreign body granulomas towards agglomerated MWCNT were also dose dependent and minimal in the low-dose group, proportional to abundance of the aggregates/agglomerates in the injected suspension. Therefore, characterization of the toxicity of single fibers needs well-dispersed MWCNT without aggregates/agglomerates. In this study, we used the recently developed dispersion method (Taquann method), which enrich the well-dispersed single fibers in the MWCNT i.p. suspension. We confirmed that the 10 μg/mice of "Taquann" dispersed MWCNT (T-CNT) induced mesothelioma in the p53+/- mice. In the Kaplan-Meier plots of the lethal mesothelioma, the slope of the p53+/- mice treated with 10 μg/mice T-CNT was allocated between the slopes of the mice with 30 and 300 μg/mice of bulk-MWCNT as shown in the previous report, e.g. in weight, T-CNT was approximately twenty times potent than the bulk-MWCNT. This increment rate in potency corresponded to the yield rate of T-CNT from the bulk-MWCNT. This finding suggested that the mesothelioma induction is dependent on the numbers of single fibers regardless of co-existing amount of aggregates/agglomerates. In addition, 10μg/mice of T-CNT induced mesothelioma in the wild type mice at a lower rate. It was incidentally found that the fibrotic response against MWCNT differed between p53+/- and wild type mice. Further studies are needed for clarifying the mechanism of mesothelioma induction and of fibrosis by MWCNT exposure. (Supported by the Health and Labor Sciences Research Grants from MHLW, Japan)

## 59. In vitro models as physiologically relevant tools to investigate pulmonary and intestinal nanotoxicity

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Establishing physiologically relevant in vitro models to investigate the response of two organs playing a key role in exposure to chemicals is of top priority therefore we develop 2 in vitro models reproducing the alveoli and small intestine barriers. These two models are more physiologically relevant, compared to the systems used so far, as there is a surfactant secretion in the alveoli system at the air-liquid interface (ALI) and a mucus production in the intestinal coculture.

The alveoli model was exposed to different realistic amounts of diesel exhaust particulate matter (80ng/cm2, or 240ng/cm2) at the ALI for different time. A clear dose-dependent translocation of the transcription factor Nrf2, which regulates gene expression in response to oxidative stress, was observed after 4h of incubation in the endothelial cells without reduction of cell viability. This show the suitability of our alveolar model in the detection of secondary induced toxicity upon realistic exposure to environmental relevant concentration of particulate matter.

The intestinal coculture model was used to evaluate effects of Ag 20 and 200 nm particles on the metabolic activity, oxidative stress and pro-inflammatory cytokine release. AgNO3 induced a reduction in metabolic activity in a dose dependent manner whereas no reduction was observed for both Ag particles. Ag was found to be homogenously distributed in the cell with aggregates observed in specific locations for Ag 20 with a 5-fold increase in IL8 release. The proteomic data revealed that both Ag particles, Ag 200 at a lesser extent, induced oxidative stress pathways and affected cytoskeleton, but regulated different sets of proteins compared to AgNO3.

Therefore, these two systems may become valuable tools for nanotoxicology in a physiologically relevant way.

## 60. Effects of Ag nanoparticles on Daphnia magna under acute and chronic tests in mineral water

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Silver nanoparticles are used more and more in consumer products, ranging from textiles, cosmetics, food packaging to electronics. This increase in their use will finally lead to their release in the environment where they will inevitably end up in aquatic environments and have a potential impact on their ecosystem. However, the fate of the Ag NPs in aquatic ecosystems is still not fully understood as well as their impacts on aquatic living organisms. For this purpose, Daphnia magna, a model species for aquatic environments, were exposed to different citrate stabilised silver nanoparticles (ci-Ag NPs: 20, 40, and 80 nm), non-citrate stabilised silver nanoparticles (Ag NPs: 20 200 nm), and silver nitrate under acute and chronic exposure conditions to evaluated their potential toxicity. In parallel to this toxicity assessment the fate of the Ag NPs was investigated by using the NTA approach. In mineral water the Ag NPs present different behaviour (agglomeration, dissolution etc.) depending on their size and surface chemistry. Indeed both Ag NPs of 20 nm formed aggregates of 100 nm whereas Ag NPs of 40 and 80 nm were more stable in exposure media. This study showed that ci-Ag NPs are more toxic in acute conditions than the non-stabilized. However, under chronic conditions a significant increase in mortality occurred when D. magna was exposed to Ag NPs of 200 nm at equitoxicity level (EC5 and EC1) compared with the other nanoparticles.

## 61. The antibacterial effects of engineered nanomaterials on Streptococcus sanguinis

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The antimicrobial properties of engineered nanomaterials (ENMs) such as silver nanoparticles (Ag NPs) or TiO2 NPs have been documented for bacteria such as Escherichia coli. However, less is known about Streptococcus sanguinis which is one of the early colonizers on the surface of dental implants, contributing to peri-implantitis. This study aimed to determine the toxicity to S. sanguinis of a range of ENMs compared to their equivalent bulk materials or metal salts, and against a positive control of chlorhexidine. Cultures of the test organism were exposed to dilutions series (400-3.125 mg/l) of each material using the minimum inhibitory concentration assay (MIC). Lactate production by S. sanguinis was also measured. The materials tested included Ag NPs, TiO2 and hydroxyapatite (HA) particles. Additions of the ENMs to physiological saline caused some aggregation. Silver nitrate was effective at growth inhibition at all the dilutions tested compared to unexposed controls. Ag NPs caused growth inhibition at 100 mg/l or less. The MIC for complete growth inhibition for TiO2 NPs was 50 mg/l, with the nano form being more toxic than a bulk TiO2. HA particles (nano or micro) were less toxic. Apparent lactate production by S. sanguinis was abolished by all concentrations of AgNO3, but only at 400 mg/l for AgNPs. The different forms of neither TiO2 nor HA had any effect on lactate production. Overall, the data shows that Ag NPs are a better antibacterial than TiO2, and that HA particles are not overtly toxic to S. sanguinus in the conditions used here.

## 62. The intracellular uptake and toxicity of functionalized Metal Oxide Nanomaterials

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Metal oxide nanoparticles (NPs) have become one of the most manufactured nanomaterials, with potential uses in a range of applications, from sensors to biomedicine. Human and environmental exposure to these NPs may however, produce adverse effects. The toxicity of copper oxide (CuO) nanoparticles conjugated to different functional groups was therefore investigated relative to their intracellular uptake.

Human bronchial epithelial (BEAS-2B) cells were exposed to positively charged core CuO and functionalised -methylammonium (-R- NH2) NPs, and negatively charged -carboxylate (-COOH) CuO NPs. Their toxicity was assessed using xCELLigence RTCA impedance based technology, and intracellular uptake was investigated using the Cytoviva® dark-field hyperspectral imaging system.

Intracellular uptake of NPs was observed for all the CuO NPs tested but the uptake of CuO-core and CuO-NH2 NPs being much higher than the CuO-COOH NPs. On the other hand, the toxicity data obtained have indicated that cells treated with CuO-core NPs and CuO-NH2 NPs had a concentration depended decrease in viability whereas cells treated with CuO-COOH NPs produced relatively no toxicity, with cells treated at lower concentrations being able to recover over time.

It can therefore be concluded that CuO NPs have shown charge dependent entry as well as toxicity to the BEAS-2B cells, with the positively charged CuO-core and CuO-NH2 NPs showing higher ability to enter the cells and also induce higher levels of toxicity compared to the negatively charged CuO-COOH NPs.

## 63. Detection of endotoxin contamination of graphene-based materials using the conventional LAL assay vs. primary human macrophages

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Detection and quantification of endotoxin or lipopolysaccharide (LPS) contamination in engineered nanomaterial (ENM) samples is a challenge, since ENM may interfere with commonly used endotoxin detection assays, e.g. the Limulus amebocyte lysate (LAL) assay. Indeed, our studies have shown that graphene-based materials (GBMs) can cause significant interference in the chromogenic LAL assay. Primary immune cells such as macrophages are exquisitely sensitive to endotoxin. Here, we devised a functional assay based on the quantification (using ELISA) of TNF-α secretion in primary human monocyte-derived macrophages (HMDM) exposed to GBMs (i.e., graphene, graphene oxide) from different sources in the presence or absence of Polymyxin B Sulfate (Poly-B), a cyclic peptide antibiotic with high affinity for LPS. Using this assay, it is possible to determine whether TNF-α secretion results from endotoxin contamination of GBMs (blocked by Poly-B) versus GBMs per se (not affected by Poly-B). The sensitivity of the TNF-α functional assay was shown to be comparable to the LAL assay. The functional assay presented here may thus provide a reliable method for endotoxin detection in GBMs. However, it should be emphasized that synthesis and handling of GBMs under sterile conditions is of utmost importance for immunotoxicity studies and for any subsequent medical use.

## 64. A self-adaptive genetic algorithm to discover minimal subsets of relevant features in OMICs data

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A class of efficient methods for searching minimal subsets of relevant features is based on Genetic Algorithms (GA). Most GA-based methods of feature selection are not well suited for high-dimensional OMICs data types such as gene expression (GE), microRNA expression (ME), copy number variation (CNV), and DNA methylation (DM), as they converge on unstable, local sub-optimal solutions. Therefore, novel adapting genetic algorithms to cope with the high dimensionality of OMICs data are necessary. We implemented a new GA-based method for feature subset selection that combines specialized genetic operators, Feature Ranking System (FRS), Fuzzy Logic Controller (FLC), Random Forest (RF) and Market Basket Analysis (MBA). The proposed method works with feature subsets (or solutions) of different length and evolves them using specialized genetic operators. The specialized genetic operators use feature ranking scores and fuzzy logic to adapt them to the specific feature selection and classification problem. The GA-process evaluates the selected subsets of features using a Random Forest (RF) and, at the end of the evolutionary process, uses market basket analysis (MBA) to select the smallest, high-accurate feature subsets from the last reached population of GA-based solutions. Our preliminary results strongly suggest that our novel method is outperforming existing ones and can address feature selection and classification tasks in the context of complex data, such as the prediction of nanomaterials safety, which we are investigating in the NANOSOLUTIONS EU FP7 project.

## 65. Time resolved dissolution study of different capped 10nm silver nanoparticles in Zebrafish Media

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Ion release kinetics dissolution of AgNPs is dependent on several environmental factors such as pH, temperature, dissolved oxygen, ionic strength, and presence of NOM as well as the physicochemical properties of the AgNPs such as its surface coating, shape, size and method of synthesis. Previous studies have assessed the effect of nanoparticle size and capping agents on the dissolution kinetics of AgNPs in a limited set of biological media. This study examines the time resolved stability and dissolu-

tion kinetics of similarly sized citrate, PVP and PEG capped AgNPs in full strength and reduced ionic strength zebrafish embryo media (ZEM) at different environmentally relevant particle concentrations. Stability studies using a variety ICP-MS and GFAAS coupled with DLS, UV-VIS and TEM show increasing agglomeration and dissolution over time. The dissolution kinetics study of citrate capped in full strength ZEM over 120 hours indicates incomplete dissolution at all particle concentrations studied, while an extended study time allowed equilibrium to be attained. The rate of silver ion release seems unaffected by concentration of AgNP in ZEM while the amount of ion released over time is concentration dependent. Comparison of the effect of two polymeric capping agents (PEG and PVP) prepared from the citrate-AgNP by ligand displacement in ZEM will also be presented.

## 66. Cytotoxicity assessment of copper oxide nanoparticles and multi-walled carbon nanotubes with amino or carboxyl surface modifications on human monocyte-derived macrophages

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Advances in nanosciences have allowed the development of a wide range of engineered nanoparticles (NPs). However, an area of concern is to ensure the safety of such nanomaterials on human health and environment. The European Commission-funded project FP7-NANOSOLUTIONS has been established to provide a means to develop a safety classification for NMs based on the understanding of their interactions with living organisms. Here we focused on the potential importance of surface modification of copper oxide (CuO) and multi-walled carbon nanotubes (MWCNTs) on cytotoxicity in human monocyte-derived macrophages (HMDMs). To this end, pristine CuO NPs and MWCNTs or CuO NPs and MWCNTs grafted with amino (-NH2) or carboxyl (-COOH) groups were dispersed and cells were exposed at concentrations up to 100 µg/mL. The effects were evaluated after 24 h using the Alamar blue assay. The NMs were first screened for endotoxin contamination using conventional methods. We detected some interference of nanomaterials in LDH assay and not in Alamar blue assay. Our studies showed that pristine CuO NPs and CuO-NH2 NPs induced more significant cytotoxicity when compared to CuO-COOH NPs. In contrast, MWCNTs with or without surface modification did not induce significant cell death. Taken together, these data have shown that different surface functionalizations can have a distinct effect on the cytotoxicity-immunotoxicity of NMs.

## 67. Genotoxicity of nanofibrillar cellulose

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Nanofibrillar cellulose (NFC) is among the most promising innovations in the forest industry. Due to its unique properties, NFC has wide-variety of application possibilities. Toxicity studies on nanocellulose materials are still scarce, and it is important to investigate the safety of NFC at an early stage of product development. The objective of the present study was to examine the potential genotoxicity of four NFC materials (fibril diameter: 2-15 nm; length: several micrometers) and a bulk-sized cellulose material.

In vitro genotoxicity was assessed in human bronchial epithelial (BEAS 2B) cells by the single cell gel electrophoresis (comet) assay (24-h exposure, doses 9.5-950 µg/ml) to detect DNA strand breakage and by the cytokinesis-block micronucleus (MN) assay (48-h exposure, doses 25-1250 µg/ml) to show possible chromosomal damage.

For genotoxicity assessment in vivo, the comet assay was performed on lung cells and bronchoalveolar lavage (BAL) fluid cells and the MN assay on bone marrow polychromatic erythrocytes, after single pharyngeal aspiration to female C57BL/6 mice (24-h and 28-d follow up; doses 10, 40, 80 and 200 µg/mouse).

The tested NFC materials did not induce significant DNA strand breakage or chromosomal damage in vitro. One of the tested nanocelluloses induced significant genotoxic effects in vivo, as determined by the comet assay 24 h after the exposure. None of the NFCs was shown to possess systemic genotoxic properties as measured by the micronucleus assay.

Our study contributes to the risk assessment of nanocellulose by providing new information about the genotoxicity of the NFC materials.

## 68. Transcriptomic signatures reflect physico-chemical characteristics of carbon nanomaterials

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Certain engineered nanomaterials (ENM) have been reported to cause adverse health effects. However, the association between ENM effects and their physico-chemical properties that lead to toxicity is still poorly understood.

The aim of this study was to investigate changes in the transcriptome patterns caused by exposure to different carbon nanomaterials (CNM) to obtain relevant information about their mode of action.

To this end, PMA-differentiated THP-1 cells were exposed to seven different CNM and asbestos for 6 and 24h and microarray analysis was performed. Our results suggest that THP-1 cells show distinct responses at different exposure times. However, carbon black (24h), long tangled carbon nanotubes (tCNT; 24h), asbestos (24h) and short tangled carbon nanotubes (SES Research; 6h) clustered together with rod-like carbon nanotubes (rCNT; 6h). An additional separate cluster including CNM with the shortest average length (SES Research, Baytubes and rCNT, 24h) also emerged. Furthermore, the data was exploited to search for gene expression signatures specifically associated to physico-chemical characteristics of the materials. Regulatory gene networks underlying the expression phenotypes were also computationally inferred.

This study provides comprehensive information about the association between relevant characteristics of ENM and their mode of action. Furthermore, the study contributes to the ongoing development of building networks related to different properties of ENM and thereby provides new information for risk assessment.

The research leading to these results receives funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement number [309329].

## 69. Inflammatory effects of CuO nanomaterials in the murine model of asthma

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Health and safety aspects of engineered nanomaterials (ENM) has been a discussion topic since the emergence of nanotechnology field. Although adverse health effects of different materials have been extensively investigated, there is a lack of information about the impact of ENM on vulnerable groups.

Asthma is a chronic inflammatory disease that develops after repeated exposure to a particular allergen. It has become one of the major childhood illnesses in Europe and in US and its prevalence is steadily increasing affecting nowadays about 5–10% of the population in developed countries.

The aim of this study is to investigate the effects of uncoated and surface modified CuO nanomaterials on allergic airway inflammation in the murine model of asthma.

In this study, mice are intraperitoneally immunized during the first sensitization period with a mixture of an allergen, ovalbumin (OVA) and an adjuvant, Alum. After 10-day recovery, mice are exposed to saline or OVA with or without dispersed CuO materials via oropharyngeal aspiration on 4 consecutive days. 24 h later blood and lung samples are collected for various analyses.

This study provides knowledge about how nano-sized CuO modulates allergen-induced asthma. Furthermore, it gives a better understanding how surface modifications affect ENM-induced responses. The data obtained in this study mimics responses of vulnerable individuals and thereby contribute to hazard assessment of ENM.

The research leading to these results receives funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement number [309329].

## 70. Inflammatory effects of nanofibrillar cellulose

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Nanofibrillar cellulose (NFC) is among the most promising innovations in the forest industry. NFC possess unique properties compared with similar materials of larger size, but it is unclear whether these properties could cause adverse health effects. Thus, it is important to investigate the safety of NFC. We examined the potential inflammatory effects of four NFC materials (fibril diameter: 2-15 nm; length: several micrometers).

Immunotoxic effects in vitro were investigated in THP-1 derived macrophages at 1, 10 and 100 µg/ml. Cytotoxicity, mRNA expression and protein secretion of pro-inflammatory cytokines IL-1β and TNF-α were assessed after 3, 6, and 24 h.

Inflammatory effects in vivo were studied in C57BL/6 mice after a single pharyngeal aspiration of 10 and 40 µg/mouse NFC. Influx of inflammatory cells, mRNA expression levels of relevant cytokines and histopathological changes in lungs were investigated 24 h and 28 days after the administration.

Two NFCs showed activation of inflammatory response. One of these induced a decrease in cell viability and up-regulated mRNA expression and protein secretion of pro-inflammatory cytokines in vitro. Furthermore, the recruitment of neutrophils and eosinophils in BAL and lung tissue, and expression of several cytokines was found in response to the NFC after 24 h in vivo. The other material did not activate macrophages in vitro, however, the effects in vivo were somewhat comparable to the ones caused by the first material. None of the materials caused long-term toxic effects.

Our study provides new information about the immunotoxicity of NFC and thereby contributes to risk assessment of these materials.

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## 71. Development of a cell line-based co-culture model to mimic the human small intestine in healthy and inflamed state

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The increasing use of nanomaterials (NMs) in food products and food contact materials urgently requires the development of reliable and efficient in vitro models allowing for thorough but feasible risk assessment to guarantee consumers' safety. Even though potentially harmful effects of orally ingested NMs cannot be ruled out to date, very little research is addressing the interaction of NMs with intestinal cells and their uptake across the intestinal barrier. The widely acknowledged limitations of current in vitro methods together with the European Union's efforts to reduce and replace in vivo experiments demand for the development of more sophisticated cell line-based models. We aimed to develop a co-culture model of human intestinal epithelial cells (Caco-2) and human macrophage-like cells (differentiated THP-1) to study the pro-inflammatory potential of silica ($SiO_2$, 17 and 100 nm) and silver (Ag, 30 nm) NMs, as well as their effects in physiological and pathological state of the barrier. We show that the condition of the barrier can be regulated by two means: the ratio of macrophages to enterocytes, and through extrinsic stimuli, such as lipopolysaccharides. The well-established co-culture model allows to study parallel intact and inflamed intestinal conditions. It enables a comparison of the effect of NMs in both healthy and susceptible individuals, which may provide viable information to policy makers and industry alike.

## 72. Mechanisms modulating the toxicity of nickel and nickel oxide particles in nano- and micron-size

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Occupational exposure to airborne nickel is associated with an elevated risk for several respiratory tract diseases, such as cancer. However, the carcinogenicity of nickel oxide or nickel metal nanoparticles has not been fully assessed. The aim of this study was to compare the toxicity (cell viability and DNA damage) of Ni and NiO particles in nano-size and micron-size, and to link the toxicity to characteristics such as oxidative reactivity, ion release and cellular uptake. Five different nickel containing particles were included in the study; nano-sized nickel metal (Ni-n) and nickel oxide (NiO-n) along with two micron-sized nickel metals (Ni-m1 and Ni-m2) and nickel oxide (NiO-m). Following exposure of A549 epithelial cells, the cell viability was decreased by Ni-n, NiO-n and Ni-m1 in a dose- and time-dependent manner in nickel concentrations from 0.1 to 40 µg/cm2 at 24h and 48h exposures.

These particles showed also the highest oxidative reactivity and released most nickel ions into the cell media. Exposure of cells to the released fraction did however not affect cell viability. NiO-n caused the most DNA damage (comet assay) at 4h in a nickel concentration of 20 µg/cm2 whereas Ni-m1 was the most potent at 24h. Cellular uptake of all particles was indicated, but the uptake of NiO-m was minimal. Taken together, the two nanoparticles tested and one of the three micron-sized particles (Ni-m1) showed the highest oxidative reactivity and metal ion release. These were also the most toxic ones indicating that particle size is not the only important parameter for toxicity.

## 73. Airway exposure to long rod-like carbon nanotubes: Comparison of oropharyngeal aspiration and inhalation methods

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Studying the pulmonary effects of engineered nanomaterials (ENM) is necessary for creating a comprehensive understanding of the ENM-induced airway inflammation. Administration of ENM by inhalation or given in dispersion by oropharyngeal aspiration are commonly used techniques for investigating pulmonary effects of the materials. However, more materials can be tested by aspiration in a relatively short period of time compared to inhalation exposures and achieving results about the possible hazardous effects is more rapid.

The aim of the study was to compare inhalation and oropharyngeal aspiration exposure techniques by characterizing both the ENM-induced inflammatory effects and the transcriptome responses in the airways.

The study comprised of exposures to rod-like carbon nanotubes (rCNT) in C57BL/6 mice. Mice were treated once, or 4 times on 4 consecutive days. 24 hours later lung samples and bronchoalveolar lavage (BAL) fluid were collected. Recruitment of inflammatory cells into airways was assessed by counting BAL cells. Total RNA from lung samples was extracted and purified for expression studies by RT-PCR and microarrays. Furthermore, lung tissue samples were also used for histopathological assessment.

Administration of ENM by aspiration revealed similar toxicological patterns as by inhalation. Secretion of inflammatory cells, especially eosinophils was seen in BAL. Moreover, common trends in the mRNA expression of pro-inflammatory cytokines (IL-1β and TNF-α) in lung tissue of mice exposed to rCNT for 4 days was seen. Genome-wide profiling by gene expression microarrays revealed complex patterns of transcriptional regulation, partially involving the alteration of similar functional pathways.

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## 74. Genotoxicity and cellular uptake of copper oxide nanoparticles in bronchial epithelial cells in vitro - effect of particle size

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The genotoxicity and cellular uptake of fine and nanosized CuO particles was studied in human bronchial epithelial cells (BEAS 2B) in vitro. Cell counting by trypan blue exclusion technique was used to determine cytotoxicity. The induction of DNA damage was assessed by the comet (single cell gel electrophoresis) assay after 3-, 6- and 24-h exposures. The micronucleus assay was applied to study chromosome damage of permanent nature.

CuO nanoparticles were clearly more cytotoxic and genotoxic than fine CuO particles, inducing DNA damage already at 2 μg/cm2, whereas a 12.5 times higher dose (25 μg/cm2) of fine CuO particles was needed to produce a significant effect. Both fine- and nanosized CuO particles induced a dose-dependent increase in DNA damage.

According to microscopic observations, CuO nanoparticles seemed to adhere tightly to the surface of the cells, whereas fine CuO particles had no such tendency, suggesting that the cytotoxic and genotoxic effects of fine- and nanosized CuO particles arise through partly different mechanisms.

The cellular uptake of the particles was investigated utilizing hyperspectral microscopy. The unique spectra of light reflected from the particles allow the recognition and mapping of the particles within the cells. The excess particles attached to the cells were removed, and the uptake was quantified by the average area covered by the particles in the cells after 1-h, 2-h and 3-h exposure. Both the nanosized and fine CuO particles appeared to be internalized by the BEAS 2B cells.

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## 75. Effect of $TiO_2$-np exposure on DNA methylation and hydroxymethylation in 16-HBE cells

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With the increase in industrial and clinical use of titanium dioxide nanoparticles (TiO2-np), a better understanding of their safety is important. In the present study the effect of different crystal phases of TiO2-np (Anatase, Rutile, and Anatase-Rutile; size range of 20-26 nm) were studied using a battery of cytotoxicity and genotoxicity assays. From the results of the cyto-genotoxicity assays, concentrations were determined for the epigenetic study. Effect on global DNA methylation and hydroxymethylation levels were studied at cyto-genotoxic (25µg/ml), genotoxic (12.5 µg/ml) and sub cyto-genotoxic (3.25µg/ml) concentrations using LC-MS/MS analysis. Though no changes were observed for 3 h treatment schedule, significant hypomethylation and changes in hydroxy-methylation levels were observed at 24 h. The lowest concentration (3.25µg/ml) showed statistically significant decrease in DNA methylation levels, comparable to the positive control (decitabine). An inverse correlation between 5-Methyl-2-deoxycytidine and 2-Deoxy-5-(hydroxymethyl)-cytidine levels confirmed demethylation induced by all three crystal phases of TiO2-np. The changes were more evident in the rutile and anatase-rutile forms. Alteration in DNA methylation pattern, including DNA hypomethylation could lead to silencing of critical genes and reprograming of cellular function. The results also suggest that epigenetic changes could occur at sub cyto-genotoxic concentrations. And hence for complete characterization of nanoparticle toxicity, epigenetic studies should be performed along with conventional toxicity testing methods.

## 76. A Genotoxicity study assessing the potential influence of metal oxide nanoparticles on the Alkaline Comet Assay

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With the expansion in nanoparticle production, many studies have focused on the cytotoxicity of these nanomaterials, with little focus on their genotoxicity. The Comet assay is one method used to assess chemical genotoxicity; concerns are however been raised on the efficacy of this assay system in accurately determining the genotoxic effects of nanoparticles. The present study investigated the possible interference of copper oxide (CuO) nanoparticles in the Comet assay system.

Human bronchial epithelial (BEAS-2B) cells were exposed to CuO NPs for an hour prior to their isolation and subsequently performing the Comet assay. In addition, modifications to the experimental design were employed, where control cells were exposed to CuO NPs after their isolation prior to performing the Comet assay. The interaction of CuO NPs with nucleosomes were also assessed using the Cytoviva® dark-field hyperspectral imaging system.

The cells incubated with the CuO NPs were shown to induce DNA damage, in a concentration dependant manner, under normal conditions. Similarly, this was observed with untreated cells 'spiked' with the CuO NPs. Although there was no incubation period with the 'spike' cells, it does allow for greater interaction between cells and NPs. The interaction between CuO NPs and the nucleotide was also observed in exposed cells following cell lysis.

The result suggest that following cell lysis, NPs are able interact with DNA causing exaggerated damage, leading to misinterpretation of false positive results. It is therefore strongly recommended that caution be taken when determining genotoxicity of nanoparticles using the Comet assay.

## 77. How many separate classes of nanomaterials are there? Results from a clustering analysis of pulmonary exposure studies in rodents

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Properties of nanomaterials may change as specific attributes are altered such as size or shape even though they remain chemically similar. Do these unique physical configurations of the same material need to be classified as categorically different substances for the purposes of toxicological risk assessment? Or, alternatively are the effects of exposure to some groups of chemically different nanomaterials so similar that they may be legitimately considered a single risk factor based on some specific characteristic such as the current designations of ambient particulates as PM10, PM2.5, and PM0.1, which classify particles only on the basis of size? This study presents a method for resolving this question based on analysis of differences in the induced effects of exposure to these nanomaterials. Through a hierarchical clustering analysis of the observed dose-response from 160 published studies involving pulmonary exposures to engineered nanoparticles in rodents, a set of effect-based statistically defined classes of nanomaterials are described. These proposed classification groupings combine nanomaterial types (defined as combinations of chemical and physical attributes) on the basis of the similarity of the observed pulmonary inflammation dose-response. The number of nanomaterial classes is a parameter defined by the tolerance of the user, such as a researcher, nanomaterial designer, or regulator, for variation in the within group dose-response. Differences between the proposed classifications on the basis of the specific observed toxic endpoint (e.g., neutrophils count or protein concentration) provide indications of present uncertainty and avenues for future investigation.

## 79. Application of short-term inhalation studies to assess the inhalation toxicity of nanomaterials

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A standard short-term inhalation study (STIS) was applied for hazard assessment of 13 metal oxide nanomaterials and micron-scale zinc oxide. Rats were exposed to test material aerosols (ranging from 0.5 to 50 mg/m3) for five consecutive days with 14- or 21-day post-exposure observation. Bronchoalveolar lavage fluid (BALF) and histopathological sections of the entire respiratory tract were examined. Pulmonary deposition and clearance and test material translocation into extra-pulmonary organs were assessed. Inhaled nanomaterials were found in the lung, in alveolar macrophages, and in the draining lymph nodes. Polyacrylate-coated silica was also found in the spleen, and both zinc oxides elicited olfactory epithelium necrosis.None of the other nanomaterials was recorded in extra-pulmonary organs. Eight nanomaterials did not elicit pulmonary effects, and their no observed adverse effect concentrations (NOAECs) were at least 10 mg/m3. Five materials (coated nano-TiO2, both ZnO, both CeO2) evoked concentration-dependent transient pulmonary inflammation. Most effects were at least partially reversible during the post-exposure period. Based on the NOAECs that were derived from quantitative parameters, with BALF polymorphonuclear (PMN) neutrophil counts and total protein concentration being most sensitive, or from the severity of histopathological findings, the materials were ranked by increasing toxic potency into 3 grades: lower toxic potency: BaSO4; SiO2.acrylate (by local NOAEC); SiO2.PEG; SiO2.phosphate; SiO2.amino; nano-ZrO2; ZrO2.TODA; ZrO2.acrylate; medium toxic potency: SiO2.naked; higher toxic potency: coated nano-TiO2; nano-CeO2; Al-doped nano-CeO2; micron-scale ZnO; coated nano-ZnO (and SiO2.acrylate by systemic no observed effect concentration (NOEC)).The STIS revealed the type of effects of 13 nanomaterials, and micron-scale ZnO, information on their toxic potency, and the location.

## 80. Comparative inhalation toxicities of graphene and other carbonaceous nanomaterials

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Graphene (G), graphite nanoplatelets (GP), carbon nanotubes (mwCNT) and low surface area carbon black (CB) are carbon-based nano-materials with broad technological applications. mwCNT and CB possess different inhalation toxicities, whereas less is known about G and GP. In order to compare the inhalation toxicity of these carbon-based nanomaterials, male Wistar rats were exposed head-nose to aerosols for 6h/day on 5 consecutive days. Target concentrations were 0.1, 0.5, or 2.5 mg/m3 for mwCNT and 0.5, 2.5, or 10 mg/m3 for G, GP and CB. Toxicity was determined at the end of exposure and three week later using broncho-alveolar lavage fluid and microscopic examinations of the entire respiratory tract. No adverse effects were observed after inhalation exposure to 10 mg/m3 GP or CB. Increases of lavage markers indicative for inflammatory processes started at exposure concentration of 0.5 mg/m3 for mwCNT and 10 mg/m3 for G. Consistent with these changes, microgranulomas were observed at 2.5 mg/m3 mwCNT and G. In order to evaluate volumetric loading of the lung as the key parameter driving the toxicity, deposited particle volume was calculated, taking into account different methods to determine the agglomerate density. However, the calculated volumetric load did not correlate to the toxicity, nor did the particle surface burden of the lung. The inhalation toxicity of carbon-based materials is likely to be a complex interaction of several parameters. Until the properties which govern the toxicity are identified, testing by short-term inhalation is the best option to identify hazardous properties in order to avoid unsafe applications or select safer alternatives for a given application.

## 82. Cerium dioxide nanoparticles protect against oxidative stress induced injury through modulation of TGF-β signalling

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Background: Cerium dioxide nanoparticles (CeONPs) have many applications including use as a diesel fuel additive. Concerns over the increased use of engineered nanoparticles and the potential risks to the public have led many studies to investigate the health impacts after nanomaterial exposure. For CeONPs, some studies report oxidative stress leading to a loss of cell viability, where others report the opposite, observing protective effects against oxidative stress induced injury. Due to a lack of consensus over the precise physiological effects surrounding CeO2 exposure, we set out to investigate how these particles influence oxidative stress induced injury.

Methods: To model the lung as a primary exposure location we used alveolar type II epithelial cells (A549) incubated with CeONPs, prior to an oxidative stress event using either H2O2 or Menadione.

Results: CeONPs exposure protected against oxidant-induced injury assessed by LDH assay. This was though not paralleled by a reduction in the oxidative stress markers such as protein carbonylation or expression of EGR1 and NQO1. Using gene expression profiling TGF-β signalling was identified as a candidate mechanism through which the CeONPs were having their protective effects. Using recombinant transforming growth factor beta 1 (TGF-β1) we were able to demonstrate the protective effects on oxidant-induced injury produced by alterations in TGF-β pathway related gene expression similar to those observed with CeONPs addition.

**Conclusions:** These results identify TGF- β signalling as a potential mechanistic regulator for the cyto-protective effects of CeONPs.

## 83. Differential long-term biodegradation kinetics within primary microglial cells of chemically functionalized carbon nanotubes

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Functionalized carbon nanotubes (f-CNTs) have been used in proof-of-concept studies to alleviate debilitating neurological conditions, such as stroke or Alzheimer's disease. Several reports have in parallel indicated that immune cells such as neutrophils, eosinophils or macrophages could participate into the elimination of carbon nanomaterials via oxidative-based biodegradation, with CNT surface properties acting as modulator of CNT biodegradability. Our previous in vivo observations suggested partial in situ biodegradation of amino-functionalized CNTs within microglia over 2 weeks, we questioned whether degradability of f-CNTs within microglia could be modulated with the type of surface functionalization used over a longer period from internalisation. The aim of the present study was therefore to investigate in isolated primary microglia the degradation kinetics of f-CNTs functionalized via different chemistries over a period of three months (90 days). Cell cultures of rat primary microglia that could be maintained for prolonged periods of time were first developed. The Raman structural signature of the internalized f-CNTs was then studied directly in cells over a period of up to three months, following a single exposure to a non-cytotoxic concentration of three different f-CNTs (carboxylated, aminated, and combined carboxylated and aminated). Structural alteration of the intracellularly-residing nanotubes suggested partial but continuous degradation over time for all nanotubes irrespective of their surface functionalization. However, carboxylation was shown to promote more pronounced modifications over the first two weeks of the study. This is the first report to investigate the long-term internalisation and biodegradation kinetics of nanostructures in primary cell (microglia) cultures.

## 84. Pulmonary acute response to separate and combined deposition of manganese and nickel nanoparticles and its attenuation with a bio-protective pretreatment

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Nickel(II) oxide and multiple manganese species [manganese (II,III) oxide included] may be jointly present in welding fumes' submicron fraction containing a lot of nano-scale particles. We prepared water suspensions of nanoparticles by laser ablation of 99.99% pure metallic plates. NiO-particles had mean±s.d. diameter 30±12nm and Mn3O4 particles - 32±10nm. Both suspensions were instilled intratracheally to rats, one immediately after another in alternating sequences or separately (another instillation being with water). The bronchoalveolar lavage fluid (BALF) was obtained 24 hrs later. The increase in total BALF cells, alveolar macrophages (AM) and neutrophil leukocytes (NL) counts and especially in the NL/AM ratio (which is an indirect but reliable comparative index of instilled particles' cytotoxicity) was significantly higher at exposure to NiO than to Mn3O4 at both 0.5 mg and 0.25 mg doses. Semi-contact atomic force microscopy of cell surface topography demonstrated active endocytosis of NiO and Mn3O4 nanoparticles by pulmonary phagocytes. The higher pulmonary cytotoxicity of NiO as compared with Mn3O4 nanoparticles was shown also by increase in lysosomal enzyme activities of the BALF supernatant. Mathematical analysis revealed a sub-additive combined action of NiO and Mn3O4, judging by cellular indices, while additivity, sub-additicty or weak synergism, judging by different biochemical indices.

In rats given glutamate, glycine, acetyl cysteine, iodide and a Se-containing multivitamin preparation orally during 4 weeks before combined exposure to NiO+Mn3O4 (0.25 mg each) the latter evoked a significantly weaker NL recruitment and thus a lower increase in the NL/AM ratio than in rats so exposed without any pretreatment.

## 85. Few-layer graphene shells and nonmagnetic encapsulates: proof of principle for a comprehensive evaluation platform to assess nanoparticle toxicity in vitro

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Nonmagnetic nanoparticles (Al2O3, TiO2, MgO) have been coated with few-layer graphene by chemical vapor deposition (CVD) of hydrocarbons. Acid dissolution of the core particles results in 3D graphene nanoshells. All particles are easily surface functionalized comparable to carbon nanotubes and planar graphene. Furthermore electrochemical investigations indicate their suitability for stable long-life battery applications.

In a precautionary approach potential adverse health effects were analyzed in parallel to the development of these novel engineered nanomaterials (ENM). Therefore we made use of an in vitro evaluation system which addresses four key aspects of cytotoxicity: viability, inflammation, genotoxicity and oxidative stress. An overproduction of reactive oxygen species (ROS) has been shown to be central for ENM induced toxicity (e.g. reviewed by Johnston et al., 2010). Subsequently damage of cellular components such as DNA, lipids or proteins could lead to cancer, inflammation and/or finally to cell death. In a first screening approach we therefore investigate ROS production (using the DCF assay) as well as acute toxicity (using the MTS assay). Besides measuring cellular responses special emphasis is put on the recognition of interference reactions of the ENM with the test systems themselves. This is mainly done under cell-free conditions.

While all few-layer graphene particles lead to a certain increase of ROS in cells as well as in a cell-free environment, no acute toxicity could be detected. Furthermore, preliminary data indicate no influence on DNA integrity as assessed by Comet assay.

## 87. Preliminary validation study of a 3D in vitro inhalation model, using cytokine and gene expression responses of copper oxide nanoparticles

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Human 3D airway models are fully differentiated and functional models of the respiratory epithelium. They are cultured at an air-liquid interface (ALI), allowing relevant air exposure. It is anticipated that these models may predict a more realistic bioavailability of inhaled compounds. To investigate the effects of donor, exposure unit, exposure session and insert, we performed air exposures of copper oxide nanoparticles using the MucilAirTM human 3D bronchial model.

MucilAirTM were exposed at ALI conditions in Vitrocell exposure modules to aerosolized CuO (0, 50, 224, 1000 mg/m3) for 1 hour. Donor and exposure module unit were rotated among the four different exposure sessions using a statistical experimental design.

Deposition of CuO nanoparticles was 4%. After a 24 hours post-incubation period, exposure to CuO showed a slight but significant LDH response for the highest dose. For inflammation markers MCP-1, IL-8 and IL-6 a dose-response was observed, where this was significant for IL-6. The influence of the parameters 'concentration' is the largest, followed by 'donor', 'unit' and 'session' which are in the same order of magnitude, which is then followed by the parameter 'insert'.

Gene expression analyses (Illumina beadchip) showed a significant increase in regulated genes (adjusted p-values <0.05) in a concentration dependent way. For the highest dose up to 5852 genes were up- and down regulated. PCA showed clearly distinct groups for 'concentration', as well for 'donor'. Statistical analyses showed that differences in 'concentration' were larger than those among 'donors', while donor differences were more substantial than differences between sessions.

We conclude that the MucilAir model can be used to assess the effects of nanoparticles, as long as donor-, session- and chip differences are taken into account of the experimental design and subsequent statistical analyzes.

## 91. Preliminary investigation of the nanotoxicity of cupric oxide (CuO) against the model microbial organism Escherichia coli

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The relative small size and unique properties of engineered nanomaterials raise concerns about their potential adverse effects on natural microbial activity, which may result from the release of these substances into the environment. The minimum inhibitory concentration (MIC) assay for growth was used to assess the antibacterial activity of pristine cupric oxide nanoparticles against Escherichia coli K12, as compared to the toxicity of the equivalent bulk CuO powder. The experiment consisted of an overnight microbial incubation at 37 oC in the presence of 100, 50, 25, 12.5, 6.25, or 3.125 mg/L of each test material suspended in sterile saline (n = 3 replicates) in 96-well microplates. The optical absorbance at 595 nm of the liquid media was used to determine bacterial growth. Afterwards absorbance values were corrected for turbidity caused by the tested suspensions in saline, and for turbidity caused by the growth medium E-basal salts with glucose. There was no observable E. coli growth under CuO nanoparticles and CuO bulk exposure, respectively, up to the lowest tested concentration of 3.125 mg/L. For the same weight per volume (100 – 25 mg/L), the nanoparticles were found to be more antibacterial towards E. coli than their equivalent bulk form. The nanoparticles displayed a clear concentration-related growth inhibition effect (r2 = 0.99); but this sensitivity was not evident from the bulk CuO toxicity data. Further testing is on-going with nanomaterials having different surface coatings. These results confirm that engineered pristine CuO nanoparticles can be more toxic to microbes than the conventional bulk form.

## 92. Dynamic coating system to modify nanoparticle surface functionality and oxidative reactivity

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Nanoparticles' (NPs) surface functionality can substantially influence their toxicity including reactive oxygen species (ROS) generation. Low volatile organic compounds (LVOC) are commonly existing air pollutants with a high affinity to surface. Previous research found that during many scenarios, such as incineration, LVOC can attach to NPs surface, resulting in modifying NPs surface function and toxicity. Thus, we built a dynamic system to simulate this phenomenon and explore how to manipulate nanotoxicity in aerosol phase. Airborne particle sizing measurement showed size growing after coating. The coating thickness can be adjusted by controlling the system parameters. Both nanotracking analyses of suspended NPs in liquid and transmission electron microscopy techniques were used to further characterize the coating and suggested the system yields stable, replicable and well controlled performance. We also observed ROS generation change, depending on the coating material and thickness. Chemical inert coating can block the reactive zones on NPs surface and decrease ROS generation; while active coating can contribute to redox circle and increase ROS generation. This study can contribute to the understanding of the effluence of NPs surface functionality on nanotoxicity.

## 93. Nanoparticles penetration through zebra fish egg membrane at the stage of perivitelline space formation

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Fish egg membrane have rather complicated structure and play an important role in embryo development protecting it from external effects. Egg membrane has pores with the diameter of 0.2-0.3 μm in zebra fish (Hart and Donnovan, 1983). The nanoparticles penetration through the non-injured fish egg membrane stayes a matter of dispute up to date. The penetration of silver nanoparticles through zebra fish egg membrane at the stage of perivitelline space formation was studied in the present work. The nanoparticles were stabilized with Ampholak 7TX- tallowamphopolycarboxyglycinate. The nanoparticles size is

10 nm. The preliminary experiments demonstrated that silver nanoparticles at the concentration of 0.1 mg/l didn't cause egg death of morphological pathology (OECD no 236). Artificial fertilization was performed in standard media, either with or without silver nanoparticles. 20 minutes later the eggs were transferred into clean media and incubated in accordance with OECD no 236. Egg death or morphological pathologies in the embryos weren't established in the control. In test group the egg death wasn't established but the morphological pathologies frequency was 40.5%. Besides, the considerable number of embryos didn't hatch and possessed morphological pathologies. In the experiment with the eggs which were placed into the nanoparticles solution after perivitelline space formation and stayed into the solution for 96 hours the morphological pathologies were not found. The embryo hatching elapsed normally. The derived data allow to assume that the stage of perivitelline space formation is critical for the nanoparticles penetration through fish egg membrane.

## 94. Toxic silver nanomaterial NM300 is detectable in macrophages by flow cytometry and decreases cellular refractive index and dry mass

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The in vitro cytotoxicity assessment of engineered nanoparticles commonly involves the measurement of different endpoints like the formation of reactive oxygen species, cell viability or cell death. Usually these parameters are determined by optical readouts of enzymatically converted substrates that often interfere with the tested nanomaterials.

Using cell viability (WST-8) and cell death (LDH) as parameter we have initially investigated the toxic effects of spherical (NM 300) and rod shaped (NM 302) silver nanomaterials with a matrix of four cell lines representing different functions: lung and kidney epithelial cells, macrophages and fibroblasts. In addition, we have used a label-free flow cytometer configuration to investigate interactions of particles and macrophages by side scatter signal analysis. Finally, we explored digital holographic microscopy (DHM) for multimodal label-free analysis of nanomaterial toxicity. Quantitative DHM phase images were analyzed for cell thickness, volume, density, dry mass and refractive index.

We could demonstrate that silver spheres lead to more cytotoxic effects than rods in all four examined cell lines and both assay. Exemplarily a dose dependent interaction increase of RAW264.7 macrophages with NM 300 and NM 302 analyzed by flow cytometry is shown. Furthermore, we found that the refractive index of cells is influenced by incubation with NM 300 in a decreasing manner. A 24 hours time-lapse measurement reveals a dose dependent decrease of dry mass and surface area development indicating reduced cell viability and death.

Our results demonstrate digital holographic microscopy and flow cytometry as valuable label-free tools for nanomaterial toxicity and cell interaction studies.

## 95. Paving the way from research to standards in the field of nanotechnologies: the nanostair support and pre-normative work

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Standardization helps capitalize, disseminate and exploit knowledge. In the controversial and rapidly evolving field of nanosafety, the efficient transfer from research to standardization is essential. Different barriers currently limit this transfer, e.g. the lack of resources after the R&D projects, and difficulties to enter the standardization process. On the other hand, researchers are eager to share their methods and have them recognized as standards.

As a response to these barriers, the FP7 cooperative action nanoSTAIR (www.nanostair.eu-vri.eu, September 2012 to February 2014) has built a sustainable process and platform to support the transfer of knowledge from research to standards in

the field of nanosafety: identifying the opportunities and the right umbrella (Technical Committee and Working Group) for standardization of research results, relying on semantic analysis and on expert review; and pooling together resources and teams on selected topics. 13 research documents have been checked by nanoSTAIR and 2 topics have been selected and are being transferred into new standards with nanoSTAIR support.

The nanoSTAIR team continues further these activities. It promotes the early implementation of this approach, from the very conception of research projects: this would allow an upfront multi-team pre-normative work on protocols within research projects and would thus dramatically facilitate the last step to standardization. The whole process will be illustrated with the case study of the new work items selected by nanoSTAIR and by the complementary case study of pre-normative work on protocols within the FP7 project QualityNano. These two case-studies together build a complete -and still ongoing- story from the start of a research project to the entrance to standardization.

## 96. Absorption, distribution and elimination of silver nanoparticles and ionic silver in rats within 24 hours after oral or intravenous exposure

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Brasseur, A., Veterinary and Agrochemical Research Centre, Belgium
Truyens, M., Veterinary and Agrochemical Research Centre, Belgium
Van der Heyden, S., Veterinary and Agrochemical Research Centre, Belgium
Mast, J., Veterinary and Agrochemical Research Centre, Belgium
Roels, S., Veterinary and Agrochemical Research Centre, Belgium

Female rats were exposed to a single dose of NM-300, consisting of silver nanoparticles with a mean diameter <20 nm, or to AgNO3. The rats were treated either by oral gavage ([Ag] = 270 and 9 mg/kgbw for NM-300- and AgNO3-exposed rats) or by intravenous injection ([Ag] = 90 or 3 mg/kgbw for NM-300- and AgNO3-exposed rats). During a 24-hour period, urine and feces were collected. After 24 hours, 18 tissues were sampled and total Ag concentrations were measured by ICP-MS. Silver was present in all rat tissues.

The absorption of Ag was <0.2% in the rats orally exposed to NM-300, while Ag excretion varied from 0.4 to 1% when the rats were intravenously treated with AgNM. Target organs differed depending on the treatment mode: pancreas and the reproductive system were targeted when orally exposed, while mainly the spleen took up NM-300 in intravenously-treated rats. Rats exposed to AgNO3, showed a higher variability in absorption and excretion than rats exposed to NM-300. The absorption varied between 0.4 and 2% of the administered Ag after oral exposure to AgNO3, while Ag excretion after intravenous exposure ranged from 19 to 36%. The pancreas was the target organ after oral exposure to AgNO3, while both liver and pancreas were the target organs after intravenous exposure.

After normalization to the Ag+-exposure dose, rats treated orally with AgNM showed a slightly higher Ag absorption than AgNO3-treated animals, indicating that a fraction of the AgNM contributed to the silver in the organs of the AgNM-group.

## 97. Distribution and biological effects of fullerene C60 after single and multiple intragastrical administrations to rats

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Due to the unique properties, the carbon nanomaterial fullerene C60 holds promise as a nano-agent in novel substances for medical applications. Investigations of its biological effects are important for assessing the risk for human health.

Study of fullerene C60 biodistribution over organs and tissues of rats after single and multiple intragastrical administrations

at daily doses of 2000 and 250 mg/kg of body weight, respectively, was carried out.

Fullerene was detected in organs of the gastrointestinal tract and in lungs on the first day of the recovery period after single exposure. On the 7th day, fullerene was found in liver, kidneys, and spleen. The highest content was observed in liver (1.21±0.11 μg/g) which was evidently the main target organ. Fourteen days after the administration, fullerene was completely eliminated from rats.

After multiple exposures we revealed fullerene accumulation in liver, kidneys, spleen, small intestine and blood serum. The highest concentration was observed in kidneys on the 7th day (1.23 μg/g). At whole, the amounts of the detected fullerene in comparison to the administered doses are far smaller that is the evidence of its efficient excretion.

No statistically significant differences in hematological and biochemical parameters of control and treated rats were found. Throughout the observation periods no lethality was observed. At necropsy, no pathomorphological changes in internal organs were recorded.

Hence, fullerene penetrates from the gastrointestinal tract into the bloodstream and translocate into secondary organs with no pronounced toxic effect in experimental conditions studied.

This study was funded by MARINA project (contract № 236215) of the EU 7th Framework Program.

## 98. The key role of microglia on interaction with chemically functionalized carbon nanotubes: implications in neurotoxicity

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Surface tunability and ability to translocate plasma membranes make chemically functionalized carbon nanotubes (f-CNTs) promising intracellular delivery systems for therapeutic or diagnostic purposes in the brain. The present study aims to determine the biological impact of different types of multi-walled CNTs (MWNTs) on primary neuronal and glial cells isolated from foetal rat frontal cortex (FCO) and striatum (ST). Neurons from both brain regions were generally not affected by exposure to MWNTs. On the contrary, the viability of mixed glia was notably reduced in ST-derived mixed glial cultures (ST-Glia), but not in FCO-derived cultures (FCO-Glia). Cytotoxicity was independent of MWNT type, suggesting an inherent sensitivity of ST-Glia compared to FCO-Glia. Interestingly, ST-Glia secreted more nitric oxide (NO) than FCO-Glia when exposed to conditioned media from MWNT-exposed microglia cultures. Characterisation of the mixed glial cultures prior to nanotube exposure revealed a higher number of CD11b/c positive cells found in ST-Glia. A higher degree of CNT uptake was also evident in CD11b/c positive cells compared to GFAP positive cells. Our results suggest that the cytotoxicity observed for ST-Glia was related to the larger number of responsive microglia contained in these primary cell extracts and emphasize the key role that resident macrophages of the brain play in response to nanomaterial exposure.

## Introducing invited speakers

Speakers are in alphabetical order.

**KARIN ASCHBERGER,** PhD
Scientific officer at European Commission
European Commission, Joint Research Centre (JRC), Institute for Health and Consumer Protection (IHCP), Systems Toxicology Unit, Italy

Karin Aschberger holds a PhD in Biochemistry and a degree in Toxicology and Nutrition Science from the University of Vienna (Austria). She has more than 15 years of experience in regulatory risk assessment of nanomaterials, chemicals, biocides and pesticides in national authorities and since 2002 at JRC-IHCP in Ispra; Italy. After 5 years in the Nanobiosciences Unit, dealing with safety assessment and regulatory aspects of nanomaterials she has recently joined the Systems Toxicology Unit where she focuses on predictive (nano)toxicology. Karin has published scientific articles and science-policy reports and is co-author of EU risk assessment reports.

**BENGT FADEEL,** MD, PhD
Professor, Karolinska Institutet, Sweden

Dr. Fadeel is Professor of Medical Inflammation Research at Karolinska Institutet in Stockholm and Head of the Molecular Toxicology Unit at the Institute of Environmental Medicine at Karolinska Institutet. He received his M.D. and Ph.D. at Karolinska Institutet and he is a Fellow of the Academy of Toxicological Sciences (ATS). Dr. Fadeel is the past coordinator of FP7- NANOMMUNE, focused on hazardous effects of engineered nanomaterials on the immune system, and his laboratory is currently engaged in several other EU-funded nanosafety projects, including FP7-NANSOLUTIONS, as well as the GRAPHENE Flagship Project. He is the current Chair of the EU Nanosafety Cluster Working Group on Systems Biology.

**DARIO GRECO,** PhD
Specialized researcher, Assoc. Prof. of Genetics, PI, Finnish Institute of Occupational Health, Finland

Dario Greco obtained the PhD in genetics in year 2009, soon after received the associate professorship (docentship) in genetics at the University of Helsinki (Finland), and currently leading the systems biology research group and the OMICs facility at the Unit of Systems Toxicology, Finnish Institute of Occupational Health. To date, he published more than 60 papers in peer reviewed journals in the field of systems biology and bioinformatics. He is leading the systems biology work package of the EU FP7 consortium NANOSOLUTIONS, where his group is developing a novel computational classifier for predicting the safety of nanomaterials.

**ANDREA HARTWIG,** PhD, Chemistry
University Professor, Food Chemistry and Toxicology
Karlsruhe Institute of Technology (KIT), Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe, Germany

Andrea Hartwig received her Diploma in Chemistry in 1984, finished 1987 her PhD thesis and 1996 her Habilitation in Biochemistry at the University of Bremen. In 1998 she became Professor for Food Chemistry at the University of Karlsruhe (TH) and 2004 Full Professor for Food Chemistry at the Technical University of Berlin. Since 2010 she is Full Professor and Chair for Food Chemistry and Toxicology at the Karlsruhe Institute of Technology (KIT). The main research area focuses on the impact of carcinogenic metal compounds including metal-based nanomaterials on genomic stability. Since 2007 she is Chair of the German MAK Commission.

**OLLI IKKALA,** PhD
Academy Professor, Aalto University, Finland

Prof. Olli Ikkala holds an Academy Professorship of Academy of Finland at the Department of Applied Physics of Aalto University (formerly Helsinki University of Technology) in Espoo near Helsinki. His research interest is in self-assembled hierarchical and biomimetic functional materials and nanocellulose. Most recent works deal interrelation of static and dynamic self-assemblies and onset of complexity. He is originally educated in physics (PhD thesis 1983 in Helsinki University of Technology on superfluid 3Helium). Then he was affiliated 10 years in chemical industry mostly related to development of polymer blends and self assembled conducting polymers at Neste Ltd. 1994 he came back to academia and established the Molecular´Materials Laboratory at Helsinki University of Technology. The laboratory has presently grown to be multidisciplinary involving ca. 30 researchers. Professor Olli Ikkala has more than 200 articles, several articles in respected journals like Science and Nature family, as well as ca. 20 patents. He has given more than 100 invited and plenary talks in international conferences. He is a recipient of the Advanced Grant of European Research Council and heads the Centre of Excellence in Molecular Engineering of Biosynthetic Hybrid Materials Research of Academy of Finland.

**KELD ALSTRUP JENSEN,** PhD
Senior Researcher, The National research Centre for the Working Environment, Denmark

Dr. Keld Alstrup Jensen obtained his PhD. in 1999 from the University of Aarhus, Denmark and spend 1½ year as a post doc at the University of Michigan, USA. He has worked at NRCWE since September 2000. He has work with full effort in the area of nanosafety since 2005 He is partner in several national and EU-funded projects. He is the key developer of the NanoSafer Control Banding tool and co-inventer of the small rotating drum under standardization in CEN under EC Mandate 461. He currently has 56 peer-reviewed scientific publications of which 42 are on nanoscale aspects.

**ANNE KAHRU,** PhD
Head of the Laboratory of Environmental Toxicology
National Insitutute of Chemical Physics and Biophysics, Estonia

Her group in NICPB was among the first ones in nanoecotoxicological studies of metal oxide nanoparticles. She belongs to the world's top 1% most cited scientists in area Environment/Ecology. In 2011, she received the Estonian State Science Award for her research "Ecotoxicology of synthetic nanoparticles and their toxicity mechanisms". She has been awarded several FP6 and FP7 research grants. Her current research focuses on the mechanisms of (eco) toxicicity and bioavailability of synthetic nanoparticles by combining molecular techniques, in vitro and ecotoxicological systems and analytical chemistry. She is also a founder (1997) and the President of the Estonian Society of Toxicology.

**GEORGIOS KATALAGARIANAKIS,** Dr.Ir.
European Commission, Directorate General for Research and Innovation, Directorate G - Industrial Technologies, Unit G4 "Nano- and converging Sciences and Technologies"

Georgios Katalagarianakis graduated as mining and metallurgy engineer from the National Technical University of Athens in 1976. He obtained a diploma on mechanical engineering from the University of Thessaloniki in 1989 and a PhD degree from Imperial College of Science, Technology and Medicine in 1998. He has worked for ten years in the underground mining industry and the mines authority of Greece before joining the European Commission in 1989 as administrator for European research in the fields of mining and metallurgy, recycling, building construction and civil infrastructure, tunnelling, industrial safety and ergonomics, etc. He is currently responsible for research in the area of nanotechnology safety and the use of nanotechnology in buildings.

**AGNIESZKA KINSNER-OVASKAINEN,** MD, PhD
European Commission, Joint Research Centre (JRC), Institute for Health and Consumer Protection (IHCP), Nanobiosciences Unit, Italy

Agnieszka Kinsner-Ovaskainen obtained her M.D. degree from the Medical University of Warsaw, Poland and the Ph.D. in Life Sciences from the University of Konstanz, Germany. She worked for 8 years at the European Centre for Validation of Alternative Methods (IHCP, JRC). In 2010 she joined the Nanobiosciences Unit (IHCP, JRC), where she is currently the leader of a Competence Group dealing with Interaction of Nanomaterials with Biological Systems. Agnieszka has over 15 years of experience in the development, validation and use of in vitro alternative methods in (nano)toxicology. She has published over 50 scientific articles in peer-reviewed journals and 5 book chapters in the area of in vitro toxicology.

**FRITZ KROMBACH,** DVM, PhD
Prof. Dr., Ludwig-Maximilians-Universität München, Germany

Fritz Krombach was promoted to full professor at the Medical Faculty of LMU Munich in 1997. He is heading an independent research group focussing on the mechanisms of leukocyte recruitment and on the fate and effects of nanomaterials in the microcirculation. Most of his R&D activity is part of interdisciplinary research projects funded by national or European research programs. Fritz Krombach is co-author or author of about 150 peer-reviewed research and review articles.

**ERIK HUUSFELDT LARSEN,** PhD
Senior researcher, Group leader, Technical University of Denmark, National Food Institute, Denmark

Erik H. Larsen is an analytical specialist with focus on toxic and essential trace elements as well as nanoparticles and their metabolites in food. Detection of nanoparticles in biological samples has been studied in vitro and in vivo. The aim of the research is to study the physico-chemical state of nanoparticles in food and their uptake, distribution, metabolism and excretion using experimental animals as model. Recently, Dr. Larsen has studied the fate of nanoparticles in the gastro-intestinal tract, based on questions regarding their solubility or possible aggregation during passage in the GI system.

**ANDRÉ NEL,** MD, PhD
Distinguished Professor of Medicine Chief, Division of Nanomedicine at UCLA
Director of UC Center for the Environmental Impact of Nanotechnology (UC CEIN), USA

Dr. Nel obtained his Medicine and Doctorate degrees at Stellenbosch University in Cape Town, South Africa, and is board certified in Clinical Immunology. He is a peer-selected member of Best Doctors of America since 1998, and received the John Salvaggio Memorial Award recognizing his outstanding service to the specialty of Immunology. He is a recipient of the Harry Truman Award for research on safe nanotechnology implementation and Nanomedicine, and has served as a panel member for President Obama's Council of Advisors for Science and Technology (PCAST) reviewing the National Nanotechnology initiative. Dr. Nel's chief research interests are: (i) Nanomedicine and nanotherapeutics; (ii) Nanobiology with particular emphasis on nanomaterial interfacial properties and quantitative structure-activity relationships; (iii) Nanotechnology environmental health and safety, with particular emphasis on predictive toxicological modeling, high throughput safety screening, and safe implementation of nanotechnology. He is Associate Editor of ACS Nano.

**HANNU NORPPA,** PhD
Research Professor, Nanosafety Research Centre and Systems Toxicology, Health and Work Ability, Finnish Institute of Occupational Health, Finland

Prof. Norppa's main research area is genetic toxicology. He has published about 200 scientific papers on genotoxicological studies of various occupational exposures, cancer risk prediction by biomarkers, individual
susceptibility, and mechanistic aspects of chromosomal damage. During the last few years, he has concentrated on nanogenotoxicology, and is presently involved in several research projects funded by the EU and domestic bodies. He has served in a number of international and domestic scientific expert groups dealing with risk assessment of chemicals and nanomaterials.

**MAURIZIO PRATO,** PhD
Professor of Organic Chemistry, University of Trieste, Italy

Maurizio Prato graduated in Padova, where he was appointed Assistant Professor in 1983. He moved to Trieste in 1992 as an Associate Professor to become Full Professor in 2000. He spent sabbatical terms at Yale University with Sam Danishefsky (1986-7) and at the University of California, Santa Barbara with Fred Wudl (1991-2). He was Visiting Professor at the École Normale Supérieure de Paris (2001) and at the University of Namur, Belgium (2010). His research focuses on the functionalization chemistry of carbon nanostructures for applications in materials science and medicinal chemistry. His scientific contributions have been recognized by National and International awards including recently: the Ciamician Gonzalez Prize, Spanish Royal Society of Chemistry (2008), the Mangini Gold Medal, Italian Chemical Society (2009), the Ree-Natta Lectureship, Korean Chemical Society (2010), the European Association for Chemical and Molecular Sciences (EuCheMS) Lecture Award (2014), the Blaise Pascal Medal for Materials Science, European Academy of Sciences (2013). He was the recipient of an ERC Advanced Research Grant, European Research Council, 2008 and has become a Member of the National Academy of Sciences (Accademia Nazionale dei Lincei) in 2010.

**MAILA PUOLAMAA,** MSc
Policy Officer, European Commission, Belgium

Maila Puolamaa has a university background in the occupational and environmental protection and she joined the European Commission as a permanent official in 2005. Her first assignment, was at Directorate General Health and Consumer Protection, working for the Scientific Committees for Newly Identified Health Risks (SCENIHR) and Consumer Protection (SCCP) with the main specialisation area nanomaterials' risk assessment. In 2008, she moved to the REACH Unit of DG Enterprise and Industry, where her main responsibilities relate to nanomaterials and the REACH contribution to the development, commercialisation and uptake of products of emerging technologies.

**BARBARA ROTHEN-RUTISHAUSER,** PhD
Full professor / Chair BioNanomaterials, Adolphe Merkle Institute, Université de Fribourg, Switzerland

Prof. B. Rothen-Rutishauser has received her Ph.D. in 1996 in cell biology at the ETH in Zurich. After a post-doctoral position in Biopharmacy at the Institute of Pharmaceutical Sciences also at the ETH she joined in 2000 Prof. P. Gehr's research group at the Institute of Anatomy, University of Bern, Switzerland. B. Rothen-Rutishauser is an expert in the field of cell-nanoparticle interactions, with a focus on lung cell culture models and microscopy techniques. Since 2011 she is the new chair in BioNanomaterials at the Adolphe Merkle Institute, University of Fribourg, Switzerland, the position is shared equally with Prof. A. Fink.

**KAI SAVOLAINEN,** MD, PhD
Director of Nanosafety Research Centre, Finnish Institute of Occupational Health, Finland

Professor Kai Savolainen is currently Research Professor and Director of Nanosafety Research Centre at the Finnish Institute of Occupational Health. His research interests cover toxicology and safety assessment of engineered nanoparticles in the occupational and general environment. He has especially conducted research on immunotoxicology of engineered nanomaterials and exposure to these materials. He has published more than 180 peer reviewed papers and served in numerous national and international scientific expert groups and has been invited to give more than 200 talks in international congresses. He has also lead several research consortia with a focus on the safety of engineered nanoparticles.

**PAUL A. SCHULTE,** PhD
Division Director, National Institute for Occupational Safety and Health, USA

Paul A. Schulte, Ph.D., is Director of the Education and Information Division, and Manager of the Nanotechnology Research Center and the Prevention through Design programs. He is an epidemiologist with training in toxicology and genetics. Dr. Schulte has conducted extensive research on occupational cancer. He is the co-editor of the textbook entitled, "Molecular Epidemiology: Principles and Practices." He has served as guest editor of the Journal of Occupational Medicine and the American Journal of Industrial Medicine and was on the initial editorial board of Cancer Epidemiology, Biomarkers and Prevention.

**ANNA A. SHVEDOVA,** PhD, DSci
Lead Research Physiologist, Adjunct Professor, NIOSH/CDC and Department Physiology and Pharmacology, School of Medicine, West Virginia University, Morgantown, WV, USA

Dr. Shvedova's lab is investigating toxicity of nanomaterials and mechanism(s) of adverse effects of nanoscale products. She has published over 150 scientific papers/book chapters in the toxicology field. She is founder/past president Dermal Toxicology Specialty Section/SOT. She was PI WP4/FW7/NANOMMUNE, and the partner of FW7/NANOSOLUTIONS. Dr. Shvedova has been honored with Awards: Public Communication/SOT; Alice Hamilton/NIOSH/best papers; NIOSH/Bullard-Sherwood-Research for Practice, Women in Toxicology/SOT. She is on board "Skin Notation"/NIOSH/CDC, Regulatory Toxicology, Associate Editor TAAP, co-editor of book "Adverse Effects of Engineered Nanomaterials". She is on advisory boards: US Army, Air Force, NASA, and the National Centre for Working Environment/Denmark.

**SIRIRURG SONGSIVILAI,** PhD
Professor, Faculty of Medicine Siriraj Hospital, Mahidol University
Executive Director, National Nanotechnology Center, Thailand

Professor Sirirurg Songsivilai is Executive Director of the National Nanotechnology Center (NANOTEC), member of National Science and Technology Development Agency (NSTDA), Thailand. He is in-charge of NANOTEC's efforts as Thailand's main driving force to establish, support and promote the development and application of national nanotechnology strategic programs through research innovations, technology transfer, human resource and infrastructure development. Prof. Songsivilai was trained in clinical medicine with M.D. degree (First Class Honours with Gold Medal) from Faculty of Medicine Siriraj Hospital, Mahidol University, and in molecular biology with Ph.D. degree from University of Cambridge, U.K. He was postdoctoral fellow at University of Colorado Health Science Center, U.S.A. In management, he received postgraduate certificates in law and public administration from King Prajadipok Institute, and in science, technology and innovation policy from Harvard University. He is certified in the Director Certification Program from the Thai Institute of Directors. Prof. Songsivilai is an Anandhamahidol Foundation Scholar awarded by H.M. the King of Thailand. He returned to Mahidol University in 1992 and, since 2000, became full Professor at Faculty of Medicine Siriraj Hospital, Mahidol University in Bangkok. His main research interest is on molecular biology and genomics of infectious diseases, especially viral hepatitis, melioidosis and avian influenza: focusing on understanding clinical characteristics from the genomics variations. His laboratory works oncutting-edge technologies including manipulation of structure of antibody molecules, discovery of new molecular targets for tropical infection and cancer, and on nanobiosensor technology. Dr. Songsivilai published extensively in international journals, authored 2 books and 5 patent applications. He received several international awards and honours, including Rockefeller Biotechnology Career Fellowship, ASEAN Young Scientist and Technologist Award, Taguchi Prize for Outstanding Achievement in Biotechnology, and National Outstanding Technologist Award. Prior to becoming Executive Director of NANOTEC in August 2008, Professor Songsivilai served as Vice President of NSTDA - Thailand's major S&T organization, overseeing management of strategic R&D initiatives, international collaborations and external affairs. He is also the Founding President of the Thailand Nanotechnology Association. Prof. Songsivilai plays active roles in various national and international networks, and is currently serving as President of the Asia Nano Forum (ANF).

**VICKI STONE,** PhD
Director of Nanosafety Research Group, Heriot-Watt University, Scotland

Vicki Stone is Director of the Nano Safety Research Group at Heriot-Watt University, Edinburgh, and Director of Toxicology for SAFENANO. She has acted as the Editor-in-chief of the journal Nanotoxicology for 6 years (2006- 2011). Vicki has also published over 130 publications pertaining to particle toxicology over the last 16 years and has provided evidence for the government commissioned reports published by the Royal Society (2003) and the on Environmental Pollution (2008). Vicki was previously a member of the UK Government Committee on the Medical Effects of Air Pollution (COMEAP) and an advisory board member for the Center for the Environmental Implications of NanoTechnology (CEINT; funded by the US Environmental Protection Agency). The nanotoxicology work at Heriot-Watt University involves funding from Research Councils (NERC, BBSRC and EPSRC), the European Commission (ITS-NANO, ENRHES, ENPRA, InLiveTox, NanoImpactNet, Marina, SUN and Qnano), charities (The Colt Foundation and The Cunningham Trust), the UK Government and industry (Unilever and GlaxoSmithKline). Vicki recently coordinated a European project to identify the work needed to develop an intelligent testing strategy for nanomaterials.

**KURT STRAIF,** MD, PhD
Head, IARC Monographs Section, International Agency for Research on Cancer, France

Dr Straif is Head of the IARC Monographs of the International Agency for Research on Cancer, WHO, Lyon, France. His research focuses on environmental risk factors for cancer. He serves on several national and international committees on primary and secondary prevention of cancer. He has a long record of teaching medicine and epidemiology and is the Scientific Director of the IARC International Summer School on Cancer Epidemiology since 2010. He studied medicine and philosophy (theory of science) at the Universities of Liège, Heidelberg and Bonn. He is Board-certified in Internal Medicine and Occupational, Environmental and Social Medicine. He received his MPH and PhD in Epidemiology from the School of Public Health, University of California, Los Angeles. He was a Research Fellow in Internal Medicine, University of Bonn, 1984- 90; Visiting Scientist, Dpt of Environmental Sciences, School of Public Health, Columbia University, New York, 1991; Assistant Professor, Institute of Occupational and Social Medicine, University of Giessen, 1991-95; Assistant and Associate Professor, Institute of Epidemiology and Social Medicine, University of Münster, 1995-2001.

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Director General, Finnish Institute of Occupational Health, Finland

Director General of the Finnish Institute of Occupational Health, Helsinki (since 2003). Dr. Vainio has over 30 years of experience at the international level from WHO and IARC. He has organized several training courses on environmental and occupational health, on biomarker epidemiology and risk assessment in developing countries in Asia, Africa and Latin America. Dr Vainio has authored over 500 research reports covering toxicology, molecular epidemiology, risk assessment, preventive medicine and occupational health. He is the Honorary Member of the Royal Colleges of Physicians, Faculty of Occupational Medicine in Dublin. Dr. Vainio served the ICOH as a board member for the tenure 2010-2012.

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### Advice to speakers

The time for free communication presentations is 15 minutes and for keynotes 30 minutes (including time for discussion). Please adhere to your allotted time as a courtesy to other speakers.

### PowerPoint presentations

Data projectors (with the PowerPoint programme) will be available, and a PC will be fixed in the meeting room for your presentation. We kindly ask you to bring your presentation with your own USB stick to the meeting room latest during the break prior to your presentation. There will be assistance in the meeting rooms to help you to do this. We strongly recommend that you have a backup of your presentation in your laptop, email or other. Please ensure that your PowerPoint version is compatible with MS Office 2013. The meeting room's laptop/pc is equipped with Windows 7.

### Briefing

Please meet the chairperson of your session 5 - 10 minutes before the beginning of the session in the meeting room and give him/her written information on how you wish to be introduced (name, title, affiliation, special interest etc).

### Poster presentation

Posters will be on display from Monday morning (13 April) to Wednesday afternoon (15 April) in the poster room Nordia (2nd floor). Please hang your poster on Monday between 8:00 – 13:30. All material needed for this will be provided. Please do not use any other materials. The authors are requested to be in the proximity of their posters during the Poster Sessions. For Poster session 1: Monday, 13 April at 13:45 – 14:45 and for Poster session 2: Tuesday, 14 April at 13:45 – 14:45. Please, check the programme for the session your poster is to be presented, and for your poster panel number.

The posters should be removed on Wednesday, 15 April between 12:30 – 13:30. The posters not removed by 15 April at 13:30 will be disposed of by the organizers/meeting venue.

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### Registration and information desk (Secretariat at the Conference site)

### Opening hours during the Congress:

Sunday 12 April, 9:00 – 18:00
Monday 13 April, 7:30 – 17:30
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### Name badges

The participants are to wear their Congress name badge at all times when at the Congress site. If you have lost your badge, please contact the Secretariat.

### Lunch

Lunch is included in the Congress registration fee.

### Certificate of attendance

A letter certifying your attendance, signed by the chairperson of the Congress Organizing Committee will be available upon request at the registration desk.

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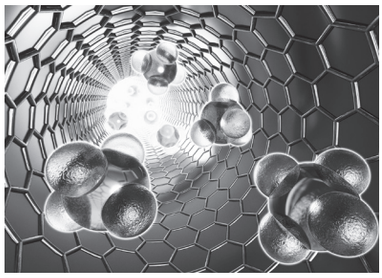

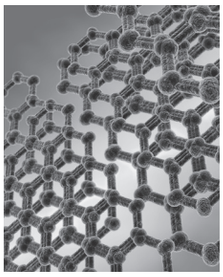

**The main innovation** of the NANOSOLUTIONS project will be the development of the engineered nanomaterial (ENM) Safety Classifier. This novel hazard profiling principle will provide a basis that enables us to understand and define the toxic potential of all types of ENM. This will allow for the crucial transition from descriptive toxicology to predictive toxicology.

Being able to effectively assess the safety characteristics of ENM will speed up the innovation cycle and the development of commercially viable products using ENM.

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NANOSOLUTIONS

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