The European Network on the Health and Environmental Impact of Nanomaterials

NanoImpactNet

Abstract Book

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For a healthy environment in a future with nanotechnology

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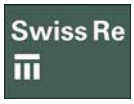

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Introduction by Professor Pierre-François Leyvraz, Director of the CHUV

Many hopes are pinned on the potential of nanotechnologies for the development of new materials and medical tools, but they also raise public concerns about possible biohazards, and they have a negative image in the context of atmospheric pollution. The scientific community takes these concerns very seriously, and in Europe as well as in North America, large studies have been initiated to investigate the issues surrounding the potential risks associated with nanotechnology, and also to find appropriate responses.

The CHUV and its associated Institutes are currently involved in large European studies aimed at the evaluation of nanoparticles and their potential effects on human health and the environment. The NanoImpactNet network in particular, funded by the European Commission in the context of the FP7 call, brings together scientists from all over Europe (over 20 countries are represented in this network). Its aim it to determine the mechanisms of interaction between the living world and human-engineered nanoparticles, along all the possible pathways of human exposition and interaction. This involves the exposure of workers involved in their manufacture, humans exposed to water or atmospheric pollution and industrial waste; but also patients for whom nanoparticles represent an immense hope for therapy and decreased side effects from treatments, particularly in cancer therapies.

NanoImpactNet is a rich network of professionals from diverse backgrounds, launched by the European Commission in April 2008 as a four-year project. From 23rd to 27th March 2009, NanoImpactNet is organizing an international conference in Lausanne on the theme of nanotechnologies and their potential impact on living organisms and the environment. This important meeting will bring together both young and more experienced scientists from the academic world, from governmental and non-governmental organizations, and from industry. This conference represents a valuable platform for the exchange of research ideas and results, and the information resulting from the presentations and discussions will be made public.

Assessing the Safety of Nanomaterials:
Recent Findings, Policy Implications, and Practical Challenges

Philippe Martin

European Commission, Directorate-General for Health and Consumers, Risk Assessment - R&D - Nanotechnologies Policy Development & Coordination

Nanomaterials attract the interest of researchers, investors, industrialists, and policy makers alike because they have something new to offer, more often than not an enabling functionalization. Unfortunately, with novelty comes a share of ignorance and the resulting need to remedy the situation. The presentation will offer a factual overview of recently published scientific opinions on the risk of manufactured nanomaterials, of current policy developments, and of some of the identified practical challenges that we must address to ensure the safe, integrated and responsible development of nanosciences and the nanotechnologies.

Session 1: Human health and exposure

Nanotechnology: The New Workplace Human Health and Exposure Issues

Challenges facing the workplace to ensure the protection of workers from occupational injury and disease resulting from the manufacture and use of engineered nanomaterials.

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Nanomaterials constitute a new generation of chemicals that exhibit behaviours not seen before in the areas of reactivity and interaction with other material systems, including biological systems. The earliest and most extensive exposures to engineered nanoparticles are most likely to occur in the workplace. Toxicological and health effects research continues to move forward in its efforts to characterize the potential hazards of nanoparticles. Recent findings indicate a prudent approach to managing engineered nanomaterials is warranted. Until more is understood about the potential health hazards and nature and extent of processes making and using these materials, current and future workers may be at risk from occupational exposures. Research studies have demonstrated greater biological activity and toxicity of nanoparticles compared to larger particles, and significant adverse effects have been shown in laboratory animal studies to be associated with some types of nanoparticles. The issue of whether to control and how to best control exposures and protect workers, given the health concerns and the uncertainties, is the basis for the current challenge. A summary of the research being conducted by the U.S. National Institute for Occupational Safety and Health (NIOSH) that has served as the foundation for the guidance document “Approaches to Safe Nanotechnology” is the focus of this presentation.

The findings and conclusions in this presentation have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy

Aggregated carbon nanotubes increase the permeability across human airway epithelium: a pathway for translocation?

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We recently demonstrated that human airway epithelial cell (AEC) monolayers exhibit increased permeability upon a prolonged exposure to multiwalled CNT (MWCNT). The impairment of barrier function seems a peculiar effect of relatively long CNT (> 5 □m) endowed with clear cut fibre properties and is not referable to marked changes in viability. To gain insight on the mechanisms underlying permeability changes, we have here used confocal laser scanning microscopy to investigate the relationships between the AEC monolayer and MWCNT. Confluent CaLu-3 cell monolayers, grown on permeable filters, were exposed for up to 8 d to 100 □g/ml of MWCNT (> 90%, iron < 0.1%; 5-9 μm length and 110-170 nm o.d.) corresponding to about 70 μg/cm^2. Once added to the medium, MWCNT formed aggregates. Calcein-loaded live AEC adhered to MWCNT tangles and, eventually, almost completely covered the bundles exhibiting the expression of the junctional protein occludin and a marked re-organization of cortical actin. After some days, the aggregates were embedded in the monolayer and gained access to sub-epithelial structures. In the contact areas several cells were caspase- and propidium iodide-positive, thus exhibiting a severe damage, while far from the tangles cells were clearly viable. However, the presence of clearly viable cells even on the MWCNT tangle suggested a continuous replacement of damaged cells.

This *in vitro* investigation demonstrates that MWCNT, even at high doses, do not cause a generalized AEC damage but, rather, focal monolayer defects. The “colonization” of MWCNT tangles by AEC resembles the “epithelial hyperplasia” observed after *in vivo* exposure to MWCNT. These data also suggest a thus far unknown mechanism to explain the entry of large MWCNT aggregates in the lung interstitium. This phagocytosis-independent translocation pathway may have important implications for the consequences of *in vivo* exposure to biopersistent, fibre-like nanomaterials.

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Workplace exposure characterisation at A TiO_2 nanoparticle production site

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The assessment of human exposure to nanoparticles needs precise information on the occurrence of nanoparticles at the workplace. As it is not defined which metric is needed for assessing the biological impact, different characteristics must be measured simultaneously. Measurements at different nanoparticle production processes were carried out. They involve the packing processes for TiO_2 like bin and bag filling, taking samples from sacked material and a comparison with outside conditions.
Parameters measured are the total number concentration for a certain size range below 1µm, the size distribution of the number concentration using a SMPS (TSI), the total surface concentration (TSI NSAM), the size distribution of the mass concentration using a low pressure cascade impactor, the size distribution of micrometer particles (TSI APS), and the mass concentrations of respirable and inhalable dust fractions according to EN 481.

Results
At the bin filling station total number concentrations between 15 000 and up to 133 000 particles per cm³ appeared (range 14-673 nm) with maxima varying between 20 and 30 nm. The higher concentrations were due to a leak and after closing the leak the concentrations decreased to less than 29 000 particles per cm^3 with maxima at 20 nm. Beyond the maxima the particle number concentration decreased steadily with increasing particle diameter up to 20 µm. The total number concentration outside the plant was approximately 13 000 particles per cm³. The inhalable dust concentration at the bin filling station was 0.232 mg/m³, the respirable dust concentration 0.10 mg/m³. Personal dust sampling for a worker in that area, who performed also different tasks, revealed 0.141-mg/m³ respirable dust concentration. On a filter surface mainly aggregates and/or agglomerates besides a few primary particles could be detected using TEM. The primary particle diameters lay between 25 and 100 nm. The main element in these particles was titanium.

Cytotoxicity of inorganic nanoparticles : role of the coating and aggregation state

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We report on the interaction behaviours between cerium or iron oxide nanoparticles and NIH-3T3 fibroblasts. The positively charged bare particles considered in this work were characterized by an average diameter 7 nm and a narrow dispersity. Different coatings were elaborated using ligands and polymers that were adsorbed onto the surface prior to the incubation experiments [1-3]. The coatings investigated were either anionic (from citrate molecules or from poly(acrylic acid) chains) or neutral (from poly(ethylene glycol) or poly(acrylamide)). Fibroblasts were incubated for 24 and 48 hours at 37° C with cerium and iron dispersions at molar concentrations ranging from 0.1 to 10 mM.

The amount of internalised particles was monitored as a function of the time by UV-spectrometry. Simultaneously, the cell confluence was determined by optical microscopy. As a result, we have found that the coating played a major role in the internalisation kinetics of the particles. The polymers tethered at the surface of the particles, either anionically charged or neutral were found to reduce considerably the uptake, and toxicity. Cell apoptose was observed in few cases, e.g. for the ligand coated particles. Another important issue about the interactions nanoparticle-cell was the state of the nanoparticles dispersion. Using new protocols for the co-assembly of nanoparticles, monodisperse clusters of size comprised between 100 and 1000 nm were designed [4-6] and incubated with the cells. A fast and significant uptake was observed for the clusters as compared to fully dispersed nanosystems. The reasons of this enhanced uptake are discussed.

1 *A. Sehgal et al., Langmuir 21 (20), 9359 (2005).*
2 *J.-F. Berret et al, J. Coll. Interface Sci. 303 (1), 315 (2006).*
3 *J.-F. Berret et al., Langmuir 23 (6), 2993 (2007).*
4 *J. Fresnais et al. Adv. Mat. 20 (20), 3877 (2008).*
5 *J. Fresnais, J.-F. Berret et al., Phys. Rev. E 78 (4), 040401 (2008).*
6 *L. Qi et al., Soft Matter 4 (3), 577 (2008).*

The oxidative potential of different carbon nanotubes *in vitro*, *in vivo*, and using a cell free system, and the effect different suspension media has on this reactive oxygen species generation.

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Although little is known concerning the toxicity of carbon nanotubes (CNT), the increasing body of literature indicates that there are definitely potential health implications associated with CNT exposure; and with increased volumes of manufacture is it vital to truly understand this health risk. The ability of CNT to cause generation of reactive oxygen species has often been shown, and may be paramount in their pathogenic behaviour, and is therefore the base of this current study.

Three structurally and compositionally different CNT (NT1 – long, straight and uniform; NT2 – short and straight; NT3 – curved and entangled), and three different suspension media, were evaluated to establish ROS generation in a cell free system, *in vitro* using a macrophage-like cell line, and *in vivo* using bronchoalveolar lavage cells.

With the time points used (30 minute and 4 hours) the entangled sample (NT3) consistently displayed a greater oxidative potential than either of the straight samples, in the cell free system; as was the case *in vitro* when cells were exposed to NT for 30 minutes. However, the cells generating ROS in response to NT1 and NT2 increased over time and were consistently significant after a 4-hour incubation period. In the bronchoalveolar lavage, cells exposed to the entangled and the short samples were found to generate significantly higher levels of ROS compared to control animals, not observed in response to the long straight sample (NT1). However, NT1 is seen to cause significantly higher neutrophil recruitment, which was not observed with the other two treatment types.

It is therefore evident that this entangled sample of CNT has, independently, a particularly high oxidative potential, and can rapidly induce ROS production in macrophages. While the straight samples produce a slow but eventual significant, and possibly prolonged, generation of ROS in macrophages; and with respect to the long, straight sample, generate an elevated immune response.

Effect of surface modification of 50nm and 100nm latex particles on uptake by, and viability of, immortal human alveolar type 1-like cells

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The rapid development of nanotechnology with the advent of nano-size materials may pose a health risk, particularly in an occupational setting. We hypothesized that particle size and surface chemistry of latex nanospheres would confer differential reactivity with human alveolar epithelial cells. Studies in our laboratory showed that human alveolar type 1-like cells (ATI) possess a significantly greater capacity for nanoparticle uptake than ATII cells. Thus, we examined the effects of 50 and 100nm latex particles (LP), with three different surface functional groups (neutral, amine-modified and carboxyl-modified), on immortal AT1 cells ((Kemp et al. 2008, 39:591-597)).

Confluent monolayers of ATI cells were incubated with 0.05, 0.5, 5.0, 25.0, 50.0 and 125μg/cm^2 LP for up to 24h. Cell viability was determined using propidium iodide uptake and cell surface annexin V staining, as well as measuring caspase 3 activity. Mitochondrial activity was measured using MTT. At 24h, neutral and carboxyl-modified LPs had very little effect on MTT, even at very high doses above 50μg/cm2, where MTT was above 80% of control. The 100nm carboxy-modified LPs had a similar effect. However, the 50nm, amine-modified LPs caused cell detachment and death, the toxic dose inducing 50% reduction in MTT activity was 41μg/cm^2. Propidium iodide uptake and annexin staining suggested both necrosis and apoptosis were responsible. Caspase 3 activity increased by 50 and 70% following 24h exposure to 50nm amine-modified LPs.
These studies demonstrate that the reactivity of latex nanoparticles crucially depends on surface chemistry and size. The exact mechanisms involved are under investigation.

Reference

1. Kemp, Sarah J., Andrew J. Thorley, Julia Gorelik, Michael J. Seckl, Michael J. O'Hare, Alexandre Arcaro, Yuri Korchev, Peter Goldstraw, and Teresa D. Tetley. 2008. Immortalization of Human Alveolar Epithelial Cells to Investigate Nanoparticle Uptake. American Journal of Respiratory Cell and Molecular Biology 39, no. 5:591-597.

Comparison of measurement devices assessing the exposure to highly agglomerated nanoparticles

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As nanoparticles are used in industry, the relevance of their health effects is discussed. Different techniques to assess workplace exposure to airborne particles are available. The goal was to compare the most commonly used devices measuring the concentration of particles <1'000nm.

Six powders (Aerosil 200 and R 974, Carbon Black FW2 and Printex 60, Aeroxide TiO2 P25 and Tego Sun T805) were dispersed with a rotating drum into a pre-filtered laminar airflow. The number concentration of particles <1'000nm was measured with three types of condensation particle counters (CPC), an optical particle counter and a diffusion size classifier, eventhough the latter is not designed to measure large powders like agglomerates.

All CPC systems as well as the optical particle counter reported similar concentrations. However the diffusion size classifier showed concentrations 100 to 1'000 times higher. The size distribution of the particles showed following geometric diameters: SiO2 (Aerosil R 974: 86nm; Aerosil 200: 228nm), Carbon Black (FW2: 436nm, Printex 60: 198nm), TiO2 (P25: 278nm, T805: 287nm). The mass concentration maximum was larger than 10 µm for all powders.

During the test chamber cleaning by a vacuum cleaner or compressed air, high concentrations of particles in the size range of primary particles were measured. For size ranges below 15nm, concentrations of >50'000 dN/dln (dp)[/cm3] were found while 1'000 dN/dln(dp)[/cm3] above 100nm.

The gentle manipulation of the powders using rotating drum and moderate airflow seems to have resulted mostly in the aerosolisation of nanoparticle agglomerates. However, the release of small (primary) nanoparticles when using vacuum cleaners or compressed air suggests that agglomerates were easily broken up or that the vacuum cleaner is a source of nanoparticles. Thus, further investigations of occupational procedures (in particular with regard to dispersive energies applied to powders) seem indicated. For industrial hygienists, information about the stability of agglomerates could be an important indicator when assessing the potential for nanoparticle liberation.

Session 2: Environmental fate and effects

Fate and effects of engineered nanoparticles in the aquatic environment

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Engineered nanoparticles (ENP) are increasingly produced and used for different applications, and their entrance into aquatic environments is therefore predictable. The assessment of associated environmental risks requires information on their behaviour and fate, their bioavailability and ecotoxicity. In spite of complexities due to the large heterogeneity of ENP with regard to chemical composition, size, and their physicochemical characteristics, principles from surface chemistry and ecotoxicology constitute basic elements to draw on for formulating predictive hypothesis that help on guiding environmental research. The surface properties of ENP are determinant of their aggregation behaviour, chemical reactivity and thus of their interaction with chemical components of natural waters. Therefore, the bioavailability of ENP will be modified according to the interactions with natural organic matter, hydrophobic organic species and metals, and the influence of ionic strength and pH.

An increasing number of publications on the ecotoxicity of ENP to aquatic organism are now appearing in the literature. Available information has evidenced a variety of biochemical and cellular effects in fish, water fleas and algae. However, interpretation of ecotoxicity data is critical in the absence of a proper physico-chemical characterization of experimental particles. ENP might display direct effects through interactions with target molecules leading to alterations of membranes and biochemical processes. Indirect effects of ENP may include physical effects, solubilization of toxic ENP compounds, or production of reactive oxygen species leading to oxidative stress. In case of engineered metal nanoparticles toxicity might relate to the occurrence of metal ions that form upon particle dissolution. Examination of the toxicity of metal ENP thus requires approaches allowing disentangling between the contribution of particles and ionic metal to the overall toxicity. Many questions regarding target organisms of ENP, the uptake and ecotoxicity thereof still remain to be elucidated before environmental risks of ENP can be assessed.

Hard protein corona Associated with NIST standard gold nanoparticles

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We have previously shown that nanoparticles in contact with a biological fluid (plasma or otherwise) interact with a range of biomolecules but in quite specified manners. [1-3] The biomolecules form a corona around the nanoparticles; it is this corona of biomolecules that govern the fate of the biomolecule-nanoparticle complex as it interacts with cells. We have characterized in depth the protein part of the corona formed around co-polymer and polystyrene nanoparticles [1-3] and shown that even for particles of the same material, but with different sizes and surface charges, the composition of the protein corona changes quite significantly. [3] This implies that extreme care must be taken in the development of nanomedicine and nanotherapeutics in terms of controlling the manufacturing process of nanoparticles and control of the surface properties of the final product.

Here we present the complete protein corona around 30 nm gold particles purchased from NIST as standard particles, along with a full detailed protocol for achieving it in a reproducible manner. We selected the NIST gold particles as they are of highest quality, are extremely well characterised, and available for all to purchase. Any lab equipped with standard biochemistry equipment can repeat the experimental protocol described here and the detection of the individual proteins can also be repeated by facilities for proteomics. The procedure and results presented here can serve as a control experiment for other groups investigating other nanoparticles protein coronas.

[1] Cedervall et al (2007) Proc Natl Acad Sci USA 104: 2050-2055.
[2] Cedervall et al (2007) Angew. Chem., Int. Ed. 46: 5754-5756.
[3] Lundqvist et al (2008) Proc Natl Acad Sci USA 105: 14265-14270.

Exposure Modelling of Engineered Nanoparticles in the Environment

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The aim of this study was to use a life-cycle perspective to model the quantities of engineered nanoparticles released into the environment. Three types of nanoparticles were studied: nano silver (nano-Ag), nano TiO2 (nano-TiO2), and carbon nanotubes (CNT). The quantification was based on a substance flow analysis from products to air, soil, and water in Switzerland. The following parameters were used as model inputs: estimated worldwide production volume, allocation of the production volume to product categories, particle release from products, and flow coefficients within the environmental compartments. The predicted environmental concentrations (PEC) were then compared to the predicted no effect concentrations (PNEC) derived from the literature to estimate a possible risk. The expected concentrations of the three nanoparticles in the different environmental compartments vary widely, caused by the different life cycles of the nanoparticle containing products. The PEC values for nano-TiO2 in water are 0.7-16 µg/L and close to or higher than the PNEC value for nano-TiO2 (<1 µg/L). The risk quotients (PEC/PNEC) for CNT and nano-Ag were much smaller than one, therefore comprising no reason to expect adverse effects from those particles. The results of this study make it possible for the first time to carry out a quantitative risk assessment of nanoparticles in the environment and suggest further detailed studies of nano-TiO2.

Preparation, Characterization and Ecotoxicological evaluation of *N-isopropylacrylamide* and *N-isopropylacrylamide-co- N-tert-butylacrylamide* Copolymer Nanoparticles.

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Poly *N*-isopropylacrylamide (NIPAM) and *N*-isopropylacrylamide/*N*-tert-butylacrylamide (NIPAM/BAM) copolymer nanoparticles of 50 to 70 nm were prepared by free radical polymerisation. The particle sizes of the copolymer nanoparticles were measured in the test media Milli-Q water, Algae Media, Daphnia Media and Microtox Diluent as a function of temperature. Whereas in Milli-Q water the particle size was seen to decrease above the lower critical solution temperature of the thermoresponsive polymer, in the test media it was seen to increase significantly, indicative of aggregation. At the temperatures employed for the ecotoxicological studies all particles, with the exception of the 50:50 copolymer existed as nanoparticles, however. The zeta potential of NIPAM and *NIPAM/BAM* copolymer particles measured in the different media was seen to correlate well with the ratio of *BAM* monomer and therefore the hydrophobicity of the particles. Ecotoxicological studies of the copolymer nanoparticles was performed using four test species *Vibrio fischeri, Pseudokirchneriella subcapitata*, *Daphnia magna*, *Thamnocephalus platyurus* and the cytotoxicity of the 100% NIPAM and 85:15 NIPAM/BAM copolymer nanoparticles was evaluated using a salmonid cell line. Although no significant cytotoxicological response was recorded, significant ecotoxicological response was observed at particle concentrations of up to 1000 mg l^{-1}. The ecotoxicological response was seen to correlate well with the ratio of BAM monomer and therefore with the zeta potential of the nanoparticles. The toxic response in *Daphnia Magna* was seen to further correlate with the reduction in zeta potential pointing towards a contribution of secondary effects due to modification of the medium. No correlation with particle size was observed. The sensitivity of the test species was seen to vary depending on co-polymer composition. The relevance of the derived structure activity relationships is discussed.

Uptake and toxicity of nanoparticles in aquatic organisms and human cell lines

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By using different test models, such as an aquatic invertebrate (*Daphnia magna*), a fish (carp; *Cyprius carpio*), primary trout hepatocytes and a human intestinal epithelium cell line (Caco-2), we assessed potential uptake of nanomaterials from water or food. We examined transport of particles through a model of the gastrointestinal barrier (M Cell model), and potential effects on the liver, using the human hepatocyte cell line C3A.

Silver particles (Ag) were purchased from Nanostructured & Amorphous materials (Houston, TX, USA) at 35 nm (nano-Ag) and 0.6-1.6 □m (micron-sized or "bulk" Ag). Cerium dioxide (CeO_2) was purchased from Sigma (Gillingham, Dorset, UK) at <25 nm (nano-CeO_2) and <5 □m (bulk CeO_2). All particles were characterised appropriately.

We found evidence of toxicity of Ag, but not CeO_2 particles in exposures of *D. magna* neonates (96 h acute exposure), primary trout hepatocytes and the human hepatocyte cell line C3A. Nano-Ag was consistently more toxic than the micron-sized silver in all models.

Uptake of Ag particles into *C. carpio* in a 21 d sub-chronic study was detected in liver, gills, gall bladder and intestine and found to occur mainly by ingestion. Ag was detected in the same organs for both particle sizes, with a trend towards higher uptake of the nano-material.

In vitro exposures resulted in uptake of both Ag and CeO_2 particles of both sizes into C3A hepatocytes, Caco-2 intestinal epithelial cells and M cells (intestinal cells specialised in transport). Our results also show the transport of these particles across the gastrointestinal barrier.

These results are relevant not only in eco-toxicology, but also in human toxicology, since nanoparticles are already in use in products designed for ingestion. The consistency of relative toxic potential of Ag and CeO_2 particles in systems as diverse as *D. magna* and human cell cultures and the size-dependent toxicity of Ag provide promising steps towards paradigms to determine toxicity of nanomaterials.

Bio-effect of nano-ZnO on *Tetrahymena thermophila**

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Several manufactured nanoparticles (NPs) are increasingly used in consumer goods. However, NPs may also have ecotoxicological effects after being discharged into the environment. Nanometer sized particles on consumer products such as nano-textiles can flow into aquatic environments, where it can exert a variety of physiologically effects in living organisms, including fish, protozoa, algae and invertebrate animals. Study of biological effects of NPs with organisms representing different trophic levels helps to understand mechanisms of uptake and following biological effects of NPs. In this study, several anorganic textile NPs (ZnO, TiO_2, SiO_2) toxicity to Tetrahymena thermophila were investigated from the cellular and molecular level and the properties of commercial nano-powder materials applied in textile products were also analysed to define the NPs-induced cytotoxicity. Laser Particle Size Analyser is used to determine the mean diameter of nano-TiO_2 and nano-ZnO in PPYS culture medium. We can conclude that nano-TiO_2 powders have aggregated so intensely in the PPYS culture medium; consequently, evaluating the aquatic toxicity of nano-TiO_2 was with no practical significance. The uptake of nano-ZnO by Tetrahymena thermophila during different time periods was observed with optical microscope and transmission electron microscopy (TEM). at various concentrations of nano-ZnO revealed that nano-ZnO of low concentrations exhibited a dose-dependent growth stimulation to the cells while the effect of 100 mg/L nano-ZnO was the most striking. Microscope images showed that food vacuoles were the main place where the nano-ZnO was distributed after took in by Tetrahymena thermophila. The viability of Tetrahymena thermophila and its dose-effect relationship at various concentrations of the NPs were measured. The result revealed that the model organism exposed to dispersed nano-ZnO solution have oxidative damages though lethal test did not give relationship data between survival and nanosuspension concentration in the laboratory condition. Among the NPs analyzed, nano-ZnO exhibited a dose-dependent growth stimulation to the Tetrahymena thermophila cells and others showed non-significant impact. The rapid proliferation of Tetrahymena thermophila cells was further confirmed by levels of the succinate dehydrogenase (SDH), protein content of control and treated groups. Our research finding provided important information on the bio-security of nano-ZnO in aqueous environment and indicated that such nanoparticles might pose potential environmental impact. Further research on the ecotoxicological mechanisms of such nanoparticles is necessary in order to minimize the adverse ecological effects and human health risk.

(1) Laura K. Adams, Delina Y. Lyon, Pedro J.J. Alvarez. Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. Water Res. 2006, 40, 3527-3532
(2) Rincon, A.G., Pulgarin, C. Bactericidal action of illuminated TiO2 on pure Escherichia coli and natural bacterial consortia: post-irradaion events in the dark and assessment of the effective disinfection time. Appl. Catal. B: Environ. 2004, 49, 99–112.
(3) Lonnen, J., Kilvington, S., Kehoe, S.C., Al-Touati, F., McGuigan, K.G.. Solar and photo- catalytic disinfection of protozoan, fungal and bacterial microbes in drinking water. Water Res. 2005,39, 877–883.
(4) Liang, J., Wu, R., Huang, T.S., Worley, S.D.. Polymerization of a hydantoinylsiloxane on particles of silicon dioxide to produce a biocidal sand. J. Appl. Polym. Sci. 2004, 97, 1161–1166.

Influence of Biopolymers and Surfactants on Dispersion of Carbon Nanotubes (CNTs) in the Aquatic Environment

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Due to their special and unique properties CNTs are very promising for industrial, commercial and environmental applications. Along with the forecasted increase in manufacturing and use of CNTs, undesired release of CNTs to the environment becomes likely. A prerequisite to assess potential effects on organisms is to know in which state, concentration and compartment the CNTs are present after their release into the aquatic environment. This project investigates how compounds present in natural waters (i.e. biopolymers and detergents) influence at environmentally relevant concentrations the aggregation and disaggregation of CNTs. Aggregation will result in sedimentation and transfer of CNTs from the water to the sediment. The characteristics of CNT suspensions containing humic acids, fulvic acids or commercial detergents were followed over time, as a function of pH and with different background ions. We introduced the CNTs in different initial states into the water (raw dry, raw suspended and acid treated (HNO_3, H_2SO_4) CNTs). These CNT-states represent conditions in which CNTs might get into the environment. Furthermore we used CNTs of different characteristics (i.e.: size, surface oxidation and purity). The mixing of CNTs with water was carried out gently by horizontal shaking and stirring, mimicking natural conditions. For comparison sonication of dispersion was done as well. The methods used for the characterization of the CNTs suspensions include TEM (transmission electron microscopy), measurements of turbidity and surface charge as well as UV-vis absorbance. The results indicate only limited dispersion of CNTs under environmentally relevant conditions. Humic acids, fulvic acids and detergents lead to the formation of small concentrations of single suspended CNTs.

Keywords: carbon nanotubes – aggregation – detergents – humic and fulvic acids

Session 3: Life-cycle and risk assessment

Setting the limits for engineered nanomaterials in the aquatic environment

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Numerous discussions during the first five years of nanoecotoxicology have been centred on the questions: Will engineered nanomaterials leach from products to the aquatic environment? and Do the European chemical regulation REACH cover engineered nanomaterials? Today it's evident that the answer to both of these questions is "yes". This answer does however pose a range of challenges to environmental assessment of nanomaterials. The passing of the Water Framework Directive (WFD) in the European Union has increased the focus on chemical compounds as potential contaminants as the water quality in receiving waters should meet goals of both good chemical and biological status. Assessments of environmental hazards related to specific engineered nanomaterials may very well be required in the future for emission to the aquatic environment event though the present regulatory focus in the WFD is set at a relatively limited number for priority pollutants. In a WFD context, environmental hazard assessments are often synonymous with comparing concentrations of priority pollutants with environmental quality standards. This presentation will explore what will happen if an engineered nanomaterial should be considered to be a priority substance in the WFD. More specifically the principles for establishing environmental quality standards for nanomaterials will be addressed in the light of uncertainties of ecotoxicological effects, persistence, and bioaccumulation of engineered nanomaterials.

Quality Assurance for Risk Assessment of Nanomaterials

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Although the main tool that scientists and regulators use to investigate the potential health and environmental risks of nanomaterials (NM) is the risk assessment framework, there are deep concerns of its applicability. Even more, the vast complexity of NM and their interactions with biotic and abiotic systems quickly leads to an overwhelming number of possible exposure routes and effects, ultimately impeding timely risk analyses and decisions by regulators, industry, and scientists. Therefore, we propose the use of an event tree (a branching system revealing interdependent relationships between parameters) to start the systematic organization and illustration of the various potential exposure pathways and human and/or environmental effects in order to ensure that all necessary factors are considered in early risk assessments. We attempt to balance the inclusion of meaningful data while minimizing the inclusion of unnecessary detail, which may obscure observed patterns or trends. Results may be used to structure discussions and increase transparency within nano-risk analyses, ultimately enhancing the quality of risk assessment of NM. Preliminary results show that, perhaps not surprising, there are hundreds if not thousands of different parameters involved in detailing potential NM risks. This highlights that we are not currently able to meaningfully conclude on the “risks of nanomaterials” as a whole given the great complexity and uncertainty involved, despite several attempts to perform preliminary risk assessments based on a few materials. Given this, we expect that risk assessment frameworks will most likely need to be revised in order to make important decisions regarding the potential risks in a timely manner based on limited information in an extremely complex world. The use of an event tree may be a first step towards achieving greater transparency in NM risk analyses, ensuring that all-important parameters are considered and the “right” questions asked.

The Significance of Nanoscale Complexity for Risk Assessment and Policy

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The fact of the scale at which nanotechnologies operate has strong implications from the point of view of complexity theory.

Factors of emergent properties, interactivity, criticality, physical self-organisation and long-term effects have so far been almost totally ignored by frontline scientists and technologists who are still operating within the inadequate conceptual framework of 19th century reductionism in which the whole is merely the sum of its parts.

Complexity theory suggests that the nanoscale requires a fundamental change of thinking in nanotechnology risk assessment and policy, in which dynamic characterisation, Life Cycle Analysis and the Precautionary Principle must have a heightened importance. The author explains why this is the case.

Hunt, G. 'Nanotechnoscience and Complex Systems', in Hunt, G & Mehta, M (eds) Nanotechnology: Risk, Ethics & Law, London: London, 2006, pp. 43-56.

Weight of evidence approach for assessing the hazard of manufactured nanoparticles: the Particle Risk results

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For assessing the health risk from exposure to manufactured nanoparticles (NPs), the EU-funded research project Particle Risk was carried out. Several physicochemical properties of tested NPs (i.e. carbon black, fullerene, carbon nantubes, nano-sized gold and quantum dots) were determined, and *in vivo* and *in vitro* bioassays were conducted to evaluate potential effects on cardiovascular and pulmonary system, as well as on liver. The integration of these results to assess the potential to cause harm for human health of tested NPs was obtained by applying a weight of evidence (WoE) approach. Based on NPs characterization activity and toxicological investigation, a preliminary set of indicators as a part of exposure and toxicity evidence has been identified and included in the WoE approach. Suitable rating values used to transform the indicator value in a qualitative hazard class have been selected for each indicator of potential exposure and toxicity on the basis of Particle Risk results, expert judgment, and literature information. A relative ranking was then calculated for tested NPs by defining a ranking procedure. Our results showed that quantum dots are the most hazardous for human health according to physicochemical characterization and toxicity data. The WoE-based ranking process offers a first procedure to assess the potential hazard of manufactured NPs taking into account the current uncertainty and data scarcity concerning the mechanisms of toxicity and exposure of NPs. This case study showed then the utility of the WoE approach and suggested that others evidences and indicators can be defined to provide conclusion about hazard of NPs as new information and data are generated.

Prospective Life Cycle Assessments of Nanosilver Textile Coatings

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Nanosilver is used in the textile industry to reduce bacterial growth. Increased use of nanosilver in textiles could result in increased human and environmental exposures to silver nanoparticles and ions. This study investigates the environmental impacts from the production and release of nanosilver, which are not thoroughly investigated to date. Life cycle inventories of two nanosilver production technologies, flame spray pyrolysis (FSP) and silver sputtering, are set up using data from an industrial and pilot plant, respectively. The inventory of the nanoparticle incorporation process into the textiles is also completed. A life cycle assessment (LCA) is conducted for a conventional versus a 'nanosilver' polyester T-shirt. Over a T-shirt's life-time the use phase can have more than ten times higher environmental impact than the nanoparticle production and coating processes (Eco-Indicator 99; IPCC 2001-GWP 100 yrs). Silver sputtering has a higher environmental impact than FSP, while both processes contribute significantly to the production phase. However, the nanosilver sputtering technique is still in a pilot phase and material and energy efficiency optimisations can be expected. During the use phase, environmental impacts can be substantially reduced by lowering the washing temperature and frequency due to the enhanced antimicrobial properties of the silver in the clothes. The disposal phase causes the least environmental impact.

A formative scenario analysis is applied to conduct a prospective LCA for Switzerland over time. Future demand is expected to be driven by public acceptance of nanotechnology in general, the effectiveness and added value of such textiles for the consumer, available technologies for production of silver-coated fabrics, and policy regulations concerning nanotechnology applications.

This study is the first, which assesses the life cycle of a nanotechnology application in the textile industry from an environmental point of view.

Hazard of engineered nanoparticles

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Although recent toxicological results highlighted the hazard associated with engineered nanoparticles (ENPs), as regard to their exposure assessment, few data are currently available, and a series of issues such as a consistent physico-chemical characterization and an appropriate toxicological experiment set-up still need to be addressed. Here we present the results obtained in the EU Project Particle_Risk. The investigated ENPs (carbon black, CB; single walled carbon nanotubes, SWCNTs; fullerene, C_{60}; nano gold, nAu; and CdTe quantum dots, QDs) have been thoroughly characterized before a complete *in vivo* toxicological experimentation was performed; the investigated ENPS were also accurately quantified after the uptake experiments. The characterization included, among the employed techniques, Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) measurements for the determination of size distribution, as well as Atomic Absorbance Spectroscopy (AAS) and Liquid Chromatography-Mass Spectrometry (HPLC-MS) based analytical methods for the quantitative uptake determination after *in vivo* experiments. The selected nanomaterials were applied to *in vivo* by intrathracheal instillation in mice. It was possible to rank the toxic effects of the tested materials: QDs, CB and SWCNT elicited a very high inflammatory potential as well as genotoxicity, while nano gold and C_{60} elicited none to low inflammatory response dependent on end-point. The obtained data permitted to obtain response-dose data useful for providing effective exposure and hazard assessment, and allowed to evaluate how size, surface chemistry and compositional properties of the ENPs may affect their ability to interact with biological systems.

Designing safe nanoproducts by combining life cycle with risk assessment

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Researchers and industrial designers aim to take advantage of the opportunities of new nanomaterials while producing safe products of high quality. Currently there are a number of major knowledge gaps in regard to the health and environmental risks posed by nanomaterials. As it would require the generation of new basic knowledge, it is unlikely that the uncertainties will be resolved in the immediate future. Nevertheless, researchers and industrial designers have to take decisions now. From the viewpoint of the uncertain effects of nanomaterials, the product will only be safe as long as no unintended release of nanomaterials nor exposure to them during the product life cycle takes place. Up to now only a few experiments have been done on the release of nanomaterials from products. Furthermore the results are difficult to interpret, as there is a lack of defined standards for measuring the release and exposure. Therefore we applied a life cycle concept in gathering information on what kind of nanomaterials could be integrated in what amount and in what way in textile products and what factors might influence the release of nanomaterials throughout the life cycle. In our study on nanotextiles, we found different integration modes as to how nanomaterials could be integrated in textiles and in what different forms nanomaterials could be released. We explain the relevant factors that may influence the exposure scenarios throughout the textile life cycle and conclude that exposure scenarios depend on the design of the specific nanotextile. With our strategy to combine the life cycle concept with risk assessment methods we intend to provide an early and holistic view of potential risks involving nanomaterials, and to contribute to informed decision making and priority setting in research, industry and regulation agencies.

Keywords: life cycle, nanomaterials, release, exposure, textile

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Stakeholder session: Risk communication and management

Risk perception of laymen versus experts and consequences for adequate risk communication regarding nanotechnology

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The Federal Institute for Risk Assessment (BfR) conducted an expert Delphi survey in 2006 on the risks of nanotechnological applications in the fields of foods, cosmetics and consumer products. The goal of this project, involving a multi-phase expert survey, was to pre-structure the technology field nanotechnology on the basis of potential risks. In the project current or potential uses of nanomaterials were identified and assigned to concrete applications. Based on the knowledge available on exposure and hazard potential, the applications were classified according to the level of probable risk. When comparing the oral, dermal and inhalation intake of nanoparticles, experts were of the opinion that the inhalation route is probably the most problematic route from the health angle. In 2006 the BfR Consumer Conference Nanotechnology was launched as part of a participatory dialogue with the public. This conference directly involved consumers in the discussions of opportunities and risks prior to the broad application of nanotechnology in society. The consumers also drew up a vote. This was the first time in Germany that a public institution had made use of this risk communication tool. The central demands of the consumers were for comprehensive labelling and accompanying risk research on 'nano' products. In order to determine how the public at large currently sees nanotechnology in Germany, the BfR conducted a research project in 2007 on public perceptions about nanotechnology. A representative population survey, combined with a basic qualitative-psychological study, sought to provide insight into the factors that influence people's perception, the social dynamics that may be of importance in conjunction with nanotechnology and the direction in which public opinion on nanotechnology could move. Risks or risk areas were identified which are present in a manifest, latent or potential manner in public perception and the impact factors for risk communication in this new risk area were described. The majority of the 1,000 respondents were of the opinion that the benefits of nanotechnology outweigh the risks (66%). They, therefore, had a good or very good feeling about this technology (77%). Whereas the use of nanotechnology in the food sector was described as a sensitive area, support in the area of textiles, paints and varnishes was high (86%). In 2008, the BfR performed an analysis of media reporting, focusing on how the subject nanotechnology is taken up in the mass media discourse, which stakeholders adopt which positions in the debate and which argumentation patterns and language images put their stamp on that debate. Nanotechnology is not a subject of great controversy in the media in Germany at the present time. Hence, it has a more positive image. The media tend to focus on the beneficial aspects in daily products like cleaning products or medical applications. Target group specific risk communication will be a reasonable way to deal with these differences in risk perception of laymen and experts.

Nano Communication in Switzerland: Perspectives from Laypeople

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In many European countries, societal communication about nano-research has been enforced by specific stakeholders. Policymakers, social scientists, NGOs, and several nano researchers advocate the integration of citizens in deliberative discussions about the development and potential risks of the emerging sciences and technologies. These stakeholders claim a dialogue between science and society to prevent risks and to avoid heavy societal controversies about nano products. Instead of informing the public "downstream", as it has happened with biotechnology when GMO products were already on the market, the "upstream" idea shared by many stakeholders aims at informing and engaging citizens at an early stage of scientific and technological innovation. This shift in science communication has lead to a remarkable number of public engagement projects in recent years. Citizen juries, discussion forums, round tables, and focus groups have been organized in a large number of countries worldwide, while most of them took place in Europe. In Switzerland, the Centre for Technology Assessment organized a so-called publifocus in which citizens discussed their hopes and concerns related to nanotechnologies. This paper looks at how citizens argued when deliberating nano-research and nanotechnologies and analyses laypeople's perspectives that emerged in this discussion forum.

The SAFENANO initiative - Proactive Risk Management for Nanotechnologies

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Since publication of the 2004 Royal Society / Royal Academy of Engineering review of Nanosciences and nanotechnologies, a small but influential body of international reviews considering the potential risks from nanotechnology have reflected a growing concern across stakeholder groups regarding potential risks to health and to the environment that may result from exposure to nanoparticles. This, in combination with recent mass upscaling of nanotechnologies in industry has led to global recognition that the safety of nanomaterials must be addressed due to their steadily increasing implementation in everyday living.

SAFENANO is a UK Government funded initiative which aims to enable industrial and academic communities to quantify and control potential risks from nanotechnology to their workforce, as well as to consumers, the general population and the environment.

Using a three-tiered approach which combines novel research, review activities, expert opinion and training, the SAFENANO initiative provides the information necessary for assessment of risks specific to nanotechnology, and facilitates responsible development of safe nanomaterials. Central to this is the initiative's maintenance of impartiality and independence of opinion.

The presentation will provide a discussion and demonstration of the SAFENANO initiative's three-tiered approach, which offers valuable tools to support risk management by those stakeholder groups working with nanotechnologies. This approach is comprised of: a website for dissemination of the latest advances in nanotechnology health and safety; a community to allow nanotechnologists worldwide to share and compare their experiences; and scientific consultancy services available to industry, academia and beyond. Via this, SAFENANO encourages proactive risk management & demonstration of responsible development in nanotechnology to stakeholder groups across the field on a global scale.

Session 5: Connecting the dots – multidisciplinary topics

Improving Nano Risk Management and Innovation Via Market-Based Approaches

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The emergent nanotechnology industry is at a crossroads, and faces significant challenges. First, the industry faces economic challenges due to increasing risk uncertainty, inefficient commercialisation, and environmental, health, and safety (EHS) externalities. Secondly, legal challenges stem from a legal framework that fails to adequately address unique nanomaterial properties and a lack of scientific research to adequately define risk and to inform more effective regulation. Thirdly, the nanotechnology industry exhibits unique market characteristics that shift traditional understanding of the innovation process and which requires tailored responses. Lastly, some current firm and market risk management practices are beginning to fail.

Current regulatory literature suffers from limited scope and market-level innovation characteristics, which leads to a focus on specific regulatory sectors, a failure to recognize private risk management as important tools, and calls for incremental change. Alternatives can better leverage maximum efficiency, risk management opportunities, and innovation.

In response, this assessment flows from a detailed and comprehensive analysis of the existing international regulatory frameworks and industry self-regulation, and includes a unique 1st-order economic analysis of all potential policy options in order to demonstrate the most efficient options. The resulting work creates a clear picture of the need for an integrated public-private regulatory framework in order to manage risk efficiently in the face of insufficient scientific data to properly develop new regulation and aggressively drive innovation. Specifically, strengthening private risk instruments, incentivising improved processes, and reducing information asymmetry all provide significant benefits by reducing risk to consumers, workers, the environment, and firms in the short- and mid-term until science can provide better risk certainty.

Risk communication and public engagement with nanotechnology

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Since 4-5 years, the emerging nanotechnology has been accompanied by a huge number of public engagement exercises, especially in Europe and in the US. More than 70 public debates or participatory procedures –citizen juries, consensus conferences, scenario workshops, etc.- have been completed. This can be considered as a specific feature of nanotechnology, and mainly a consequence of the GMO controversy. The GM crops saga is held by public actors as an exemplary failure in the way a new technology has been introduced in society, especially in Europe, mainly attributed to a communication deficit at an early stage of the technology's trajectory. The idea is to prevent a similar backlash for nanotechnology by fostering an "upstream public engagement". Aside of stakeholders' dialogues, participation and hearings are among the best ways to acknowledge the concerns of citizens, and take it into consideration in designing and marketing products. New technology raises uncertainties, and new risk must be studied carefully in order to build trust. In this paper, we analyse the importance of an inclusive approach of risk, and of public participation for risk communication and management by public and private actors. We provide an overview of the risk governance and communication best practices, and a preliminary assessment of current achievements.

Promoting Good Practices for Handling Nanomaterials-An International Wiki Project

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As production and use of nanomaterials in commercial products grow it is imperative to ensure these materials are used safely with minimal unwanted impacts on human health or the environment. Foremost among the populations of potential concern are workers who handle nanomaterials in a variety of occupational settings, including university laboratories, industrial manufacturing plants and other institutions. Knowledge about prudent practices for handling nanomaterials is being developed by many groups around the world but may be communicated in a way that is difficult for practitioners to access or use. The GoodNanoGuide is a collaborative, open-access project aimed at creating an international forum for the development and discussion of prudent practices that can be used by researchers, workers and their representatives, occupational safety professionals, governmental officials and even the public. The GoodNanoGuide is easily accessed by anyone with access to a web browser and aims to become a living repository of good practices for the nanotechnology enterprise. Interested individuals are invited to learn more about the GoodNanoGuide at http://goodnanoguide.org.

The Analysis of Nanomaterials in Food

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The potential benefits for consumers and producers of the application of nanotechnology are widely recognized. Products based on nanotechnology or containing engineered nanomaterials (ENMs) are already manufactured in the field of electronics, consumer products and pharmaceutical industry, and are beginning to impact the food associated industries. ENMs are claimed to be used in the entire food chain, e.g., during cultivation (agriculture), industrial processing, and incorporated into food packaging materials. The current usage levels of ENMs in the food and feed area is unknown, but given the claims indirect consumer exposure to ENMs cannot be excluded.

Detection and characterization of ENPs is an essential part of understanding the potential benefits as well as the potential toxicity of these systems in food. However, the development and application of analytical methods for the determination of ENMs in different matrices, and certainly in food, is still in its infancy. During the last year we developed an analytical method for the determination of silver ENMs in food matrices and are planning to expand this method to inorganic ENMs of different composition. The method is based on a combination of hydrodynamic chromatography for size separation, coupled with ICP-MS for element specific detection of silver ENMs, and preceded by a sample preparation method to isolate ENMs from the matrix. This presentation gives a short overview of available analytical methods for detection and characterization of inorganic ENMs, followed by the choices we made and the results obtained during the development, application and limited validation of the analytical method.

Evidence maps - a tool for dealing with Uncertainties
Summarising and communicating evidence in the assessment of data uncertainties and the practical application in the field of engineered nanoparticles

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The pool of scientific data within a particular biological remit, e.g. nanotoxicity, is often complicated by the limited quantity and inconsistency of the associated findings. This may lead to uncertainties when assessing current state of the art knowledge thus, ultimately, compromising current and future research efforts.

Based on the development of a novel tool dealing with such uncertainties when evaluating the biological impact of electromagnetic fields, the “NanoHealth” project, which is funded by the Helmholtz-Society in Germany, aims at dealing via the novel “evidence map” approach with the health impact of nanoparticles.

When fully implemented, this approach would enable to handle the limited or inconsistent scientific data associated with certain hazards. Such noxious matter includes most nano-scale materials and their possible biological effects.

The evidence maps strive for providing a better tool for risk assessment. Moreover, this approach aims at establishing a specific communication network whereby contradictory findings, scientific views and expertise can all be exchanged amongst stakeholders in this field. The evidence map method was adopted as a potent evidence assessment tool comprising a transparent and unambiguous graphical presentation. This is based on the following elements of this evidence weighing process; (i) the database (number of relevant scientific studies); (ii) the debated possible relationship between exposure to a specific (potential) hazard and the associated biological effect and; (iii) the conclusions based on such debate and the remaining uncertainties. These elements together form the basis of the evidence map. We have implemented this tool to generate evidence maps for various nanomaterials and different biological reactions/endpoints. The outcome of this approach, and the associated evidence maps, were discussed in several working groups with experts, stakeholders and the general public.

Consumer exposure to household sprays containing nanoparticles: Experimental Assessment and Modelling

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Nanoparticles are already on the market in various consumer products from different industrial branches. Those products include household sprays used for cleaning, impregnation and surface treatment of various materials such as leather, textiles, wood or glass. Nanoparticles are spray ingredients because of their advantageous antimicrobial, antifungal or UV-filtering properties.

At the same time, toxicological studies have shown that nanoparticles may have negative health effects when reaching the lungs. However, in order to accurately assess the risk to consumers, toxicological data have to be combined with exposure modelling.

In this study, we have modelled the exposure of consumers to nanoparticles in sprays on the basis of experimental data. Different sprays in propellant gas and pump spray vessels were investigated and compared to a blank with respect to size distribution and nanoparticle concentration. The size distribution in the course of the spraying process was determined by scanning mobility particle sizer (SMPS). The concentrations of nanoparticles in the spray solutions and the sprayed aerosol were analyzed by inductively coupled plasma mass spectrometry (ICPMS). The morphology and particle size were determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

The modelled exposure levels provide a basis for a realistic consumer risk assessment concerning nanoparticle-containing household sprays.

Poster Presentations

Validation of aerosol measurement technologies for in- and outdoor use

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Our studies will show the objective of bringing into light and to document the need for standardised test aerosols adapted to the scope of nanotoxicology and occupational health studies. State-of-the-art nanoparticle generation and aerosol monitoring equipment will be used to show that the properties (such as size, size distribution, surface chemistry, surface charge, concentration) of aerosols formed by manufactured nanoparticles experience dramatic changes when released in the ambient air compared to their initial properties within the reactor. The project will provide introductory experimental results that will be used.
The target is to develop recommendations and guidelines for the users worldwide. New validation methods particularly adequate to test aerosols suitability for filtration and respiratory protection will be shown We will show validation methods with comparisons tests and other results.

The aim of is to help define realistic test conditions in terms of test aerosols characteristics (particle size distribution, concentration and surface chemistry) for use within nanotoxicology investigations but also to test and certify the efficiency of existing engineering control systems of manufacturing equipment (such as air cleaning systems, personal protective equipment).

An overview about the continuous methods for nanoparticle production (aerosol process) will be prepared to show which industries are involved in that field and which production methods are used to give a first impression of the different production methods and production conditions. This could be e.g. flame process, spray process, milling or ablation process in different temperature, pressure and concentration conditions. This would give a first feeling on critical exposure situations to the workplace and the environment and relevant scenarios will be developed.

Development of methods for testing the toxicological profile of nanoparticles used in the biomedical field.

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Nanoparticles are becoming a very interesting option for both medical diagnosis and targeted drug delivery. Their large biomedical application in the future is probable, however detailed studies have to be conducted in order to exclude any potential toxicity to living organisms. Our objectives are to evaluate the interaction of different types of nanoparticles with specific cells, their cell uptake and release, their transport across biological barriers and their potential cytotoxic effects. This information will then be useful to design efficient standardized *in vitro* methods for testing various nanoparticles of potential future interest. In a first approach we have studied Superparamagnetic Iron Oxide Nanoparticles (SPION) coated with amino-PVA, which are taken up by human cells and whose uptake is enhanced by an external magnetic field. These SPIONs were able to be transported across human colon CaCo2 cells and brain-derived HCEC endothelial cells, as models of biological barriers, depending on their concentration and the presence of the external magnetic field. They did not show any significant cytotoxicity at doses which can be foreseen to be used *in vivo*.

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Cell imaging using up-converting phosphor nanoparticles

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Up-converting phosphors, which emit visible light upon near infrared excitation, have potential in biological labelling and imaging applications. The purpose of this project is to prepare sensitive up-converting nanoparticles of defined size for biological studies. $NaYF_4$ has been reported to be the most efficient host material for green/red up-conversion phosphors when doped with Ytterbium and Erbium (Krämer et al. 2004, 1244). $NaYF_4$:Yb,Er nanoparticles have been prepared in the aqueous phase. We will discuss details of the nanoparticle preparation as well as some properties of these nanoparticles including their morphology, photoemission yield and structure in relation to parameters of their preparation. Optical images of these up-converting nanoparticles following contact with cells in culture will also be presented.

Karl W. Krämer, Daniel Biner, Gabriela Frei, Hans U. Güdel, Markus P. Hehlen, and Stefan R. Lüthi. 2004. Hexagonal Sodium Yttrium Fluoride Based Green and Blue Emitting Upconversion Phosphors. Chemistry of materials 16(7): 1244-1251.

Present address: Paul Scherrer Institut, Laboratory of Atmospheric Chemistry, CH-5232 Villigen, Switzerland

Characterization of cell death induced by Silver Nanoparticles using flow cytometric and microarray analysis

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Silver nanoparticles have been tested concerning their toxicity on different mammalian cell lines. With focus on the possible adsorption of the nanoparticles into the human organism, via skin and via respiratory tract, the effects on fibroblasts (NIH-3T3) and on a human lung adenocarcinoma epithelial cell line were examined. Additionally, the particles were tested with HEP-G2 cells, which are often used as model cell line for biocompatibility tests, with PC-12 cells, a rat adrenal pheochromocytoma cell line and with CACO-2 cells, a human colon adenocarcinoma cell line. The viability of the cells was examined by the MTT-test.
Cytometric flow measurements to determine apoptosis or necrosis were also performed with NIH-3T3 cells treated with the silver nanoparticles.
For analysing changes in gene expression associated with the silver nanoparticles treatment, DNA microarray analyses on silver nanoparticles treated HEP-G2 cells were performed. The viability results were found to partly depend on the type of cells used. It could be shown that the silver nanoparticles show a significant suppression of the cell growth.

Comparison of two redox reactivity tests on different nanoparticles

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Biological effect of nanoparticles (NP) appear to be related not only to surface or size but also to their ability to generate oxidants, either intrinsically or through cell activation. Such an oxidative capacity for a NP panel has been determined by measuring their ability to catalyse the electron transfer between a reductant (dithiothreitol – DTT) and oxygen.
In order to confirm the reactivity order found with the DTT test, the oxidative capacity of a NP panel (three black carbon, two diesel particulate and two TiO_2) was determined with two different reactivity tests:
1. the DTT assay [Sauvain et al.,2008]
2. by measuring the oxygen consumption in a closed system when a suspension of NP in put in contact with an ascorbic acid solution [Pan et al., 2004]

We also tried to characterise some of the surface functional groups present on the NP in order to understand which chemical functions could play a role in such a reactivity. Surface functional groups were chemically probed with different gases, using a Knudsen cell.
For the selected carbonaceous NP panel, both reactivity tests gave very similar mass-based reactivity order with FW2 being the most reactive and Printex 60 the less. Expressing the reactivity-results on a per-surface basis changed the order with the two diesel samples being the most reactive ones. However, both methods suggested again a similar order of surface-based reactivity.

A rather good correlation between the mass-based reactivity and the acidic surface content of the carbonaceous NP was observed, suggesting that in presence of reductants (DTT or ascorbic acid), oxidised carbonaceous NP are able to catalyse the electron transfer to oxygen in a more efficient way than NP in a more reduced state.

Sauvain JJ., Deslarzes S., Riediker M., Nanotoxicology 2008, 2(3) :121-129
Pan C., Schmitz D., Cho A., Froines J., Fukuto J., Toxicol Sci 2004, 81(1):225-232

Cytotoxic effect of ZnO nanoparticles on Human Colon Carcinoma cells (LoVo)

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Aim of this study was to investigate the role of the physico-chemical properties of ZnO nanoparticles (ZnO-NPs) in the potential cytotoxicity and oxidative stress induction on a human colon carcinoma cell line (LoVo). As negative control, "fine" TiO_2 particles were used. The structural characterization of particles, suspended in the culture medium, was carried out by scanning electron microscopy using a Soft Imaging System. Both ZnO and TiO_2 particles showed a quite regular globular shape. The mean diameter of ZnO-NPs ranged from 38 to 964 nm with an average of 196 nm. 34% of particles possessed dimensions below 100 nm. The mean diameter of TiO_2 particles ranged from 47 to 1862 nm with an average of 396 nm. Only 15% of them were smaller than 100 nm. To evaluate if ZnO and TiO_2 particles induced effects on cell growth and cell survival, count of viable cells after trypan blue staining and cell death evaluation by flow cytometry were performed on LoVo cultures exposed to different particle concentrations (15, 30, 45 and 90 μg/ml) for 24, 48 or 72 hours. Cells responded to ZnO-treatment with significant growth inhibition, particularly evident at the higher doses (45 and 90 μg/ml), already after 24h of exposure. The growth curves of LoVo cells treated with TiO_2 did not show any significant dose and time dependent effect. Treatments with ZnO-NPs induced at high concentrations and exposure times (48 and 72h) remarkable cytotoxicity. On the contrary, cultures exposed to TiO_2 particles showed cell death percentages similar to those of control cells. In addition, ZnO-NPs induced a significant increase of cytosolic ROS after 48h of exposure. Also TiO_2 particles raised ROS content, already after 24h, but the cytotoxic effect was not revealed. These results demonstrate that ZnO-NPs induce significant cytotoxicity on LoVo cells. Moreover, the dimensional analysis seems to suggest that particles with diameter <100 nm and not the larger ones are mainly responsible of cell damage.

Increased lung toxicity in animals exposed to Zinc Oxide nanoparticles by inhalation: A dose response relationship by surface area

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BACKGROUND: Although adverse respiratory effects of ZnO nanoparticles (NPs) have been studied, data on the dose-response relationship for inhaled ZnO NPs toxicity are limited.
AIM: To evaluate the dose-response relationship of ZnO NPs on lung toxicity and systemic inflammation, and use the Benchmark dose to assess the reference dose for ZnO NPs on these effects.

METHODS: Healthy SD rats were randomly divided into four groups: control (filtered air; n=12), low dose group (n=6), moderate dose group (n=6), and high dose group (n=6). ZnO NPs produced in a furnace were used to treat animals by inhalation in a whole body chamber for 6 hrs. SMPS was used to monitor particle size and number, and the toxicity was assessed by enumeration of total cells, determination of total protein and LDH activity in BALF. Complete blood counts in peripheral blood were also determined. Then, the Benchmark dose (BMD) software was used to assess the BMDL.

RESULTS: Results showed that the count median diameter of ZnO NPs for low-dose, moderate-dose, and high-dose group were 37.7 nm, 37.9 nm and 35.6nm, respectively. And the estimated surface area concentrations of each group were 1.3*104, 2.5*104, and 1.0*105mm2/m3. In all the three exposed groups, polymorphonuclear leukocytes (PMN), total cells, total protein, LDH and WBC increased significantly compared to the controls ($P<0.05$). Further, dose-dependent increases in PMN were observed ($P<0.05$). The BMD modelling gave BMDL of surface area-based dose of ZnO NPs was estimated at 10745mm2/m3, which is equal to mass-based dose of 1.07 mg/m3 at 36nm of ZnO.

CONCLUSIONS: Our results have demonstrated increased lung injury and systemic inflammation in animals exposed to ZnO NPs by inhalation. Further, results from our assessment suggest the current US OSHA standard for ZnO fume (PEL, 5 mg/m3) may not be sufficient to protect workers against lung inflammation.

Assessment of lead exposure due to ingestion and inhalation of particles emitted from workplaces in a lead-recycling plant.

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The lead-recycling plants are currently one of the human main activities emitting lead in the environment. Their employees are exposed to lead-rich particles from process emissions trough two ways of exposure: inhalation and ingestion.

Fine particulate matter (PM_{10}), ultrafine ($PM_{2.5}$) and nanoparticles produced at workplaces contribute to the exposure of workers: they firstly represent the inhalable fraction and probably contribute to a significant fraction of the ingested particles. For ingestion assessment, in addition to the total metal content in particles, it is important to know the bioavailable lead fraction. Bioavailibility can be approximate by the bioaccessible fraction (fraction of the contaminant solubilised in the gastro-intestinal tract). To our knowledge, the presented study is the first to report on lead particles bioaccessibility in occupational environment. Concerning inhalation, particle impact seems to depend of intrinsic physical properties (size and specific surface) and also of chemical properties. In particular, it has recently been advanced that oxidative properties of particles induce the generation of reactive species of oxygen (ROS), which could be at the origin of biological effects observed following their inhalation.

The objective of this work was to study the influence of the particle size on the lead bioaccessibility (BARGE[1] test, norm ISO ISO/TS 17924) and redox activity (DTT[2] assays). Lead bioaccessibility is function of the sampling site and increases as particles size decreases. The presence of lead particles with DTT indicated a tendancy to stabilise it with no clear size dependency. It contributes to the application and the validation of these two chemical tests which could become tools for evaluation of the potential health hazards. Such tests could be useful in the frame of the European REACH legislation.

[1]BARGE: Bioaccessibility Research Group Europe
[2] DTT: dithiotreithiol

Surface and bulk properties of SiC and TiC nanoparticles after dispersion in biocompatible solutions for toxicological assessment

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Nanoscience and nanotechnology are highly promising areas for research and industrial innovations. Due to their unique properties, manufactured nanoparticles are of great of interest for many industrial and medical applications. However, there is a growing concern for their possible environmental and health impacts. Information about the specific characteristics of nanoparticles, such as morphological, chemical and physical properties, is critical when trying to understand and assess their potential risks.

The unique nature of nanoparticles expressed in their size, shape and surface characteristics is expected to play an important role in their possible toxicological effects. Aggregation and agglomeration phenomena are frequently observed when nanoparticles are dispersed in biocompatible solutions for *in vivo* and *in vitro* toxicology tests. The surface of the nanoparticles is the first to interact with the dispersion media, tissue or cellular wall. Therefore a proper knowledge of its chemical composition is of prime importance, particularly knowing that this composition can be modified in contact with the host media.

Our work is related to a detailed characterization of the surface properties of nanoparticles (SiC and TiC), before and after dispersion in biocompatible solutions such as pluronic F108 surfactant.

In addition to the solubility influence, the results demonstrate drastic changes in surface's chemical composition: on one hand, some nanoparticules are wrapped by a coating related to the dispersion medium, and on the another hand, nanoparticle's extreme's surface could be dissolved, exposing the inner layer to the transporting solution which in turn interacts with the cells directly. Therefore it is important not to disregard the effects of the dispersing media on the nanoparticles themselves when considering their toxicological effects.

The results discussed are based on XPS, TEM, SEM and EDX analyses.

A new simple instrument for real-time monitoring of airborne nanoparticles

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During the handling of nanomaterials there is the risk that nanoparticles are released in airborne state, and these nanoparticles might be harmful to health when inhaled by the employees. Thus, monitoring of nanoparticle concentrations is recommendable, and the Scanning Mobility Particle Sizers (SMPS), which employs a Differential Mobility Particle Sizer (DMA) and a Condensation Particle Counter (CPC), constitutes the standard instrument for measuring size and concentration. While such instruments provide a complete size distribution even for very low number concentrations, they offer however a limited time resolution and they are rather large and elaborate to operate.

We have developed the simple portable instrument “Nanocheck”, which is intended for fast and easy assessment of nanoparticle exposure risks. The instrument consists of a corona charger, a condenser, and a Faraday Cup Electrometer, and it measures total number concentration and mean size of nanoparticles. The system features a time resolution of 6 s, a detection limit of some 500 particles/ccm, a size range of 25 – 400 nm, and it is attached to a portable optical aerosol spectrometer to provide also number and mass concentrations for larger particles. Thus, not only nanoparticle concentrations are monitored, but also the well established values for inhalable, thoracic and alveolic mass fraction can be determined with a single portable system. Comparison of the Nanocheck with the SMPS system as a reverence showed an excellent agreement both for total number concentration and mean particle size.

REACH Regulation and Safety Assessment of Nanomaterials. Studies Using Nano-Titanium as a Model Agent.

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Nanotechnology is expected to provide great benefit along with potential risks for consumers, workers, and the environment. In order to ensure that a high level of protection is maintained, one of the challenges is to verify that current legislation(like REACH Regulation)could cover the risks associated with nanomaterials.

REACH is based on the principle that the manufacture or use of chemicals do not adversely affect human health or the environment.

Federchimica(the Italian Federation of the chemical industry),with the collaboration of the University of Pavia and Colorobbia Italia SpA, set up a project with the aim of collecting toxicological information onto nanomaterials and developing guidelines for safe uses of nanomaterials complying to REACH.

Nanostructured titanium dioxide(TiO_2)produced by Colorobbia has been selected as a model material, given its technological importance and its disparate applications from cosmetics to additives.

A first step was data collection from public databases and specific SDS (Safety Data Sheet) analysis. Information from more than 1000 documents was evaluated in relation to the endpoints necessary for a Chemical Safety Assessment(CSA).

The results indicated great differences between the nano and macroforms of TiO_2 in terms of physicochemical and toxicological properties. The available data are also incomplete, often conflicting and not sufficient to cover all the endpoints requested.

A data gap analysis was then conducted to determine which data are to be produced for the nanoform CSA.

The next step will be the implementation of a database with all the toxicological info collected, the definition of specific Exposure Scenarios and related Risk Management Measures. Special attention will be given on aspects related to consumer safety and environmental dispersive products.

The ended study should help identifying new testing protocols and strategies that can be used to produce a complete CSA for TiO_2-based nanomaterials according to REACH requests.

Neuronal cells response onto patterned nanostructured substrates

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The interaction of nanostructured materials with living systems is an emerging research field which is attracting increasing interest, due to the ability of nanomaterials to trigger specific responses. Moreover, the investigation of nanomaterials toxicity, along with several important perspectives in tissue engineering and regenerative medicine, are crucial for the development of a wide range of therapeutic and diagnostic applications. However, the mechanisms underlying cells interactions with nanostructures at the molecular level remain poorly understood. The combination of nanofabrication techniques with biological sciences may provide interesting tools to achieve a precise control of the cellular microenvironment and to evaluate the mechanisms and spatiotemporal aspects of nanomaterial interactions with living systems. In this work, we show the behaviour of human neuroblastoma cells (SHSY-Y5 cells) onto patterned gold substrates with periodic flat/nanorough features, obtained by a combination of wet-chemical and lithographic techniques. High resolution imaging by confocal and holographic microscopy revealed important morphological and functional differences in response to the nanoroughness of these substrates. We observed events of specific cell adhesion, axonal autogrowth, axonal pathfinding and cytoskeletal specific orientation only in the smooth areas of the substrates, resulting in a clear self-alignment of neuronal cells. This study suggests that neurons are extremely sensitive to the substrate topology and that cellular adhesion machinery senses environmental cues and responds to them, possibly opening novel concepts into nanostructure-triggered biological responses.

NANOMMUNE: Comprehensive assessment of hazardous effects of engineered nanomaterials on the immune system

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Despite the tremendous opportunities of engineered nanomaterials there are considerable knowledge gaps concerning the potential hazardous effects of ENs on human health and the environment. The NANOMMUNE partnership aims to fill these gaps through a comprehensive assessment of ENs, with particular focus on effects on the immune system.

The main project objective is to study the possible hazardous properties of engineered nanomaterials on the immune system. The partners in the consortium have a complementary expertise in a wide variety of areas. Interdisciplinary collaborations are required to fully understand the properties and biological effects of nanomaterials. Our network consists of experts in material sciences, cell biology, toxicology, immunology, systems biology and risk assessment. This allows for interdisciplinary design, execution, interpretation and translation of research which is needed to address some of the major issues around the emerging nanotechnologies. We aim to analyse and predict the toxic potential of ENs on key functions of the innate and adaptive immune system such as particle uptake and immune regulation. In addition, detailed physico-chemical characterization of ENs is an integrative part of the project. Overall, the NANOMMUNE project results will enhance the understanding of possible adverse effects of nanomaterials and will contribute to a continuous and sustainable growth of the nanotechnologies. Partners in the project aim to share their findings in peer reviewed publications, presentations, popular scientific articles and a quality handbook with the collected laboratory protocols.

The NANOMMUNE project was launched on September 1st 2008 and will run for 3 years. The project has a total budget of € 3.358.500 and is funded by the European Commission through the 7th Framework Programme by the funding scheme of Collaborative projects, in the area of Nanosciences, Nanotechnologies, Materials and New Production Technologies (NMP4-SL-2008-214281) .

Determination of the lung-deposited nanoscale particle dose

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Health risks associated with the production and use of nanoparticles are not yet fully understood. Particularly airborne exposure and inhalation is currently seen as the major route of uptake. The characteristics relevant for nanoscale particles induced health effects are still not known but different mechanisms are currently discussed. Experts contend that particle surface area or number concentration may show higher associations to health outcomes than the currently used indicator mass. It was suggested that the determination of the lung deposited dose may be a better health-relevant value than airborne concentrations. Lung-deposition is determined by the exposure concentration and the specific particle properties, size and hygroscopicity, that determine percentage and location of lung deposition (Löndahl et al. 2007). The dose can then be assessed with breathing volume and frequency (Asbach et al. 2009). Newly developed instruments (NSAM/Aerotrak 9000, TSI) determine surface area concentrations ($\mu m^2/cm^3$) of particles that would deposit in the tracheobronchial and alveolar region of a reference worker during medium activity according to the ICRP model (ICRP, publication 66, 1994). These kind of instruments may therefore be very relevant for research in the fields of inhalation toxicology, health effects and workplace exposure by enabling dose assessments. The NSAM/Aerotrak is currently calibrated for lung deposition curves of a reference worker, however, it can be re-calibrated to mimic almost any personal lung deposition curve. If breathing rate and tidal volume of that specific worker are also known, the personal surface area dose of the inhaled particles can be derived. Otherwise average breathing rates and tidal volumes can be used to estimate a generic dose. This approach allows tailored assessments of a personal health risks of a worker associated with the inhalation of nanoscale particles. Possibilities to realize personal dose monitors will be presented along with some preliminary results.

Löndahl, J., A. Massling, J. Pagles, E. Swietlicki, E. Vaclavik, S. Loft (2007): Size-resolved respiratory-tract deposition of fine and ultrafine hydrophobic and hygroscopic aerosol particles during rest and exercise. Inhal Tox. 19: 109-116.

Asbach, C., Fissan, H., Stahlmecke, B., Kuhlbusch T.A.J., Pui, D.Y.H. (2009): Conceptual limitations and extensions of lung-deposited Nanoparticle Surface Area Monitor (NSAM). J Nanopart Res 11: 101-109.

Short and long term toxicity effects of colloidal nanosilver in the zebrafish animal model

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Silver nanoparticles possess a widely known antimicrobial effect with a broad field of application especially in medicine and pharmacology. Recently, the exploitation of such properties by the Food Industry has been envisaged. As a matter of fact, nanosilver is already employed for antimicrobial purposes as a coating material in some type of food packages as well as in some food related appliances, such as refrigerators. A number of studies investigating the safety of nanosilver have pointed out possible toxic effects of this molecule in some cell lines and in some aquatic organisms. Our studies have the objective of defining short and long term effects of colloidal nanosilver exposure on an entire organism using the zebrafish animal model. We carried out *in vitro* tests on zebrafish embryos to mimic acute toxicity tests and analysed morphological parameters as well as the toxicogenomic response of some specific toxicity biomarkers. In parallel we carried out an *in vivo* test by exposing zebrafish larvae to a variety of colloidal nanosilver concentrations analysing possible developing and morphological alterations during their growing period up to the adult stage. According to our preliminary results, nanosilver exposure causes morphologic changes at the embryonic stage; changes in the expression level of most of the toxicity biomarkers tested, and some developmental alterations. The effects detected do not always reflect a dose dependent scheme. Silver ions were also tested as a comparison and did not show the toxicity levels of nanosilver. The results obtained so far indicate that before considering the application of nanosilver in food related fields, its effects on vertebrates should be exhaustively determined.

Development of *In Vitro* Tests to Measure Transport and Toxicity of Nanoparticles to Placental Function: NanoTEST

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The objective is to examine the toxicity and transport mechanisms of a range of well-defined and medically relevant nanoparticles (NPs) in models of the human placental barrier, in order to estimate the extent of potential NP damage and transfer to the fetus following human exposure.

The human *in vitro* placental BeWo cell line is seeded on permeable Transwells® to generate monolayers. Development and optimisation has produced a robust model as determined by confocal and EM, tight junction antibody staining, and transport marker permeability. Barrier integrity is determined by TEER measurements which must fall within a defined range. Preliminary experiments show that latex Fluoresbrite® NPs (40-60 nm) can cross the placental barrier and other NPs (including TiO_2, paramagnetic metal oxides, metal fullerenes, polylactic glycolic acid and quantum dots) will be evaluated, with permeability measurements enabling direct comparison of NPs.

A range of assays will be used to evaluate the cytotoxic and genotoxic effects of NPs on placental function. These include DNA damage as determined by Comet and micronucleus assays; viability assays including WST-1 and LDH assays; differentiation/proliferation assays such as BrdU and hCG production; oxidative stress and ROS production and pro-inflammatory assays including cytokine mRNA production and cytokine secretion. Assay optimisation has been completed for LDH and WST-1, and validated using cobalt-chrome NPs (29 nm). The WST-1 assay has shown a BeWo dose response when exposed to CoCr NPs (0.005-0.04 mg/mL) for 24hr.

Developmental work is underway to establish a more *in vivo*-like model of improved relevance. Co-cultured cell populations such as endothelial cells (HUVEC) will be used for an assessment of the effects of co-culture on parameters evaluated in the monolayer model.

Comparison of the pulmonary toxicity of micro and nano sized gold particles in rats

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Introduction: Due to physical characteristics such as size, surface reactivity and surface to volume ratios, the interaction of nanoparticles versus micro sized particles of the same material with biological systems might be markedly different. Colloidal gold was chosen as a model particle to study effects on systemic and lung inflammatory markers after intratracheal instillation. The study focuses on the potential toxicity of gold particles of 10 and 250 nm in the lung.

Methods: A single dose of 200 µg/kg body weight of gold particles in aqueous suspension of 10 nm and 250 nm (SPI supplies), diluted 10% by adding 10x concentrated phosphate buffered saline were administered to male WU Wistar-derived rats by intratracheal instillation. A single dose of 200 µg/kg body weight and 1000 µg/kg body weight DQ12 quartz was used as a positive control. Dynamic light scattering (NanoSight) revealed the particle size distribution in the suspensions prior to application. Transmission electron microscopy (TEM) analysis of particles solutions and interaction with cells from broncho alveolar lavage fluid (BALF) was performed. BALF and blood was collected to assess the effect on cell differentials, oxidative stress and inflammation after 19 and 72 hrs.

Results: In both 10 nm and 250 nm solutions, small and larger aggregates as well as single particles were found. Single and aggregated 10 nm and 250 nm particles were detected inside macrophages. A significant but small increase compared to the positive control quartz in monocytes and IL-6 levels is observed in the lung 72 hrs after 250 nm gold application. This was not evident for 10 nm gold particles. After 19 hrs, an oxidative stress response is seen in the lung using 250 nm, but not with 10 nm particles. No sign of systemic inflammation by gold particles is seen.

Conclusion: After intratracheal instillation, 250 nm particles elicit a mild inflammatory response in the lung, whereas 10 nm particles did not.

Integrating LCA and EHS expertise in the assessment of nanoparticles

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DTU with their expertise in LCA have joined forces with IOM in two nanotechnology-related projects, one of which additionally involves and is lead by CSL. The first project, Nancore, includes the evaluation of health risks and environmental impacts over the life cycle concurrently with the development of new production technology for lightweight materials used in e.g. wind turbine blades. As the new technology involves the use of nanoparticles, the health and safety workpackage is needed to examine the potential exposure and effects of these particles. An LCA is also performed to evaluate the overall environmental impacts of the technology. Through the concurrent assessments, the strengths of the two tools are combined to provide a more qualified assessment of both the health and safety aspects and the life cycle impacts.

The overall aim of the second project, sponsored by Defra, is to identify products containing CNT and evaluate the potential for inhalation exposure of a representative subset of products. As part of this study, we are investigating how a standardized Life Cycle Assessment can contribute to the analysis. Simplified LCAs will be performed for a few products. In particular, the issue of improving the estimates of health impacts across the life cycle, through the concurrent exposure and health effect analysis, will be addressed.
The presentation will introduce the approach underpinning our approach in the Nancore project and present preliminary results from the second project.

Probing the Potential Carcinogenity of Carbon Nanotubes

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Carbon nanotubes (CNTs), due to their distinct needle-like structure, similar to asbestos, have been suggested as potentially carcinogenic (Donaldson *et al*., 2006). Recently, CNTs have been shown to induce granulomas *in vivo* (Poland *et al*., 2008), as well as mesothelioma in the peritoneal cavity (Takagi *et al*., 2008). Despite this, the ability of CNTs to cause cancer within the lung is not fully understood, regardless of the potential for increased inhalation of these nanoparticles during their production and use within a range of consumer and industrial applications. The aim of this project was to determine the ability of well-characterised and well-dispersed bundles of single-walled CNTs (Wick *et al*., 2007) and multi-walled CNTs (Wick et al. under revision) to cause cancerous mutations in the lung. In addition, both asbestos (crocidolite) fibres and diesel exhaust particles, a classified 2A human carcinogen (Bao *et al*., 2006), were used as positive controls. Using a triple co-culture *in vitro*-model, which has been shown to mimic the epithelial airway barrier of the lung (Rothen-Rutishauser *et al*., 2005), initial investigation into the cytotoxicity of all particles and fibres was determined via the MTT assay and the release of lactate dehydrogenase from lung cells. Additional study also consisted of an assessment of the mitochondrial membrane potential of lung cells following exposure to particles and fibres, as well as investigation into the potential process of cell death via FACS analysis (Annexin V/Propidium Iodide staining). The potential mutagenicity of all particles and fibres was subsequently determined via the use of the comet assay and the Ames test. In addition, the potential for CNTs to cause oxidative stress was investigated by determination of reactive oxygen species. No data can be reported at this time, as the project has only recently commenced. Representative data will be available for presentation at the NIN integrating conference.

References:
Bao L, Chen S, Wu L, Hei TK, Wu Y, Yu Z, Xu A. 2007. Mutagenicity of diesel exhaust particles mediated by cell-particle interation in mammalian cells. Toxicology 229: 91-100.
Donaldson K, Aitken R, Tran L, Stone V, Duffin R, Forrest G, Alexander A. 2006. Carbon nanotubes: a review of their properties in relation to pulmonary toxicology and workplace safety. Toxicology Sciences 92: 5-22.
Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WAH, Seaton A, Stone V, Brown S, MacNee W, Donaldson K. 2008. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nature Nanotechnology 3: 423-428.
Rothen-Rutishauser B, Kiama SG, Gehr P. 2005. A three-dimensional cellular model of the human respiratory tract to study the interaction with particles. Am. J. Respir. Cell Mol. Biol. 32: 281-289.
Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J. 2008. Induction of mesothelioma in p53+/- mouse by intraperitoneal application of multi-wall carbon nanotube. Toxicology Sciences 33 (1): 105-116.
Wick P, Manser P, Limbach LK, Dettlaff-Weglikowska U, Krumreich F, Roht S, Stark WJ, Bruinink A. 2007. The degree and kind of agglomeration affect carbon nanotube cytotoxicity. Toxicology Letters 168: 121-31.

Alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics. NanoTEST – EC FP7 project

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The unique properties of nanoparticles (NP) whilst likely benefit many aspects of our life, are also the cause of concern over inadequate toxicological assessment of their possible impact on human health. The area of nanomedicine brings humans into direct contact with NP and appropriate risk assessments in relation to health and safety is essential for both nanotech companies and public confidence. The NanoTEST project (*www.nanotest-fp7.eu*) addresses these requirements in relation to the toxicological profile of NPs used in medical diagnostics.

The overall aim of NanoTEST is to develop alternative testing strategies and high-throughput toxicity-testing protocols using *in vitro* and *in silico* methods which are essential for detailed risk assessment. NanoTEST specifically aims to: a) carry out a detailed characterization of selected NPs to define main describing physico-chemical properties, b) study specific and non-specific interactions of NPs with molecules, cells and organs and develop *in vitro* methods for identifying toxicological potential of NPs, c) validate *in vitro* findings in short-term *in vivo* experiments in ethically approved models, d) perform structure-activity modelling and physiologically-based pharmacokinetic (PBPK) modeling of NPs, e) adapt the most advanced and promising assays for high-throughput automated systems and to prepare them for validation by the European Centre for the Validation of Alternative Methods (ECVAM). A common database will be developed and available for all partners and used for NP characterization, *in vitro*, *in silico* (QSAR, PBPK modeling) and *in vivo* assays.

Development of *in vitro* models using cell lines and cells from several different organs is in progress together with optimizing protocols for biomarkers of oxidative stress, inflammation, immunotoxicity, cellular toxicity and genotoxicity. Preliminary results will be presented.

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Nanoparticles, animal welfare and human protection: the need to reassess safety testing

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The use of manufactured nanomaterials raises health concerns due to their unknown toxicity. The applicability of existing animal-based safety tests being used to assess nanomaterials has been questioned on numerous occasions. Current safety testing protocols carried out for nanoparticles do not represent the most scientifically applicable, robust and consistent methods. They rely heavily on time-consuming animal methods which are not designed around the specific properties of nanomaterials and will cause an unnecessary increase in the use of animals in research and compromise human because the animal tests are not able to provide regulators or companies with relevant or reliable safety data.

In the absence of validated nano-specific testing strategies and whilst nano-specific methods are being developed, we recommend a battery of *in-vitro* test methods to be employed and required from regulators for nanoparticles. In addition, there is already a wealth of human data available which can be extracted from the population already exposed to nanomaterials. Human-relevant *in-vitro* assays offer several advantages: using human cells or sub-cellular components they avoid species differences, and high-throughput systems allow the very rapid and cost-effective testing of multiple chemicals. There are a number of available *in-vitro* techniques that can be developed for use with nanomaterials. For example, human cell culture techniques, perfusable 3D cell-matrix chambers, lab-on-chip technology and computer modeling techniques.

We suggest that in the absence of a battery of tests covering all the relevant human-health related a series of validated and OECD accepted non-animal test methods should be compiled and companies required to submit data using those methods. For additional human health effects assessment, we suggest a tiered, weight-of-evidence approach based on the most relevant methods available at this time.

Characterisation of Nanoparticle Size and State Prior to Nanotoxicological Studies

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Before commencing any nanotoxicological study, it is imperative to know the state of the nanoparticles to be used, and in particular their size and size distribution in the appropriate test media. Particles satisfying standards can be commercially purchased, however these invariably cannot be used directly; and need to be dispersed into the relevant biological media. Often such changes in the environment or ionic strength, or a change in the particle concentration, results in a level of aggregation or a shift in the particle size distribution. Such unexpected aggregation, dissolution or plating out, if unaccounted for, can have a significant effect on the available nanoparticle dose and on interpretation of any results obtained thereafter.

Here we demonstrate the application of characterisation instrumentation that sizes nanoparticles based on their Brownian motion in suspension. Unlike classical light scattering techniques, the Nanoparticle Tracking and Analysis (NTA) technique allows nanoparticles to be sized in suspension on a *particle-by-particle* basis allowing higher resolution and therefore better understanding of aggregation than ensemble methods (such as dynamic light scattering, DLS).

Results are presented from gold (standard) nanoparticles in biologically relevant media that emphasise the importance of characterisation of the nanoparticle dispersion. It will be shown how the NTA technique can be extended to multi-parameter analysis, allowing for characterization of particle size and light scattering intensity on an individual basis. This multi-parameter measurement capability allows sub-populations of nanoparticles with varying characteristics to be resolved in a complex mixture. Changes in one or more of such properties can be followed both in real time and *in situ*.

Assessment of occupational exposure to Diesel exhaust particles in association with levels of oxidative stress biomarkers

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Exposure to fine and ultrafine particles is associated to many adverse health effects (respiratory and cardiovascular diseases, cancer). A hypothetical mechanism to explain these effects is the ability of components (organics, metal ions) adsorbed on these particles to generate Reactive Oxygen Species (ROS), and thereby to cause oxidative stress in biological systems. ROS can attack almost any cellular structure (DNA, cellular membrane), leading to the formation of a wide variety of degradation products which can be used as biomarker of oxidative stress. If the level of ROS increases, a defence mechanism among others induces the production of antioxidants to counter ROS [1].

The aim of the present research project is to test whether there is a correlation between the exposure to Diesel Exhaust Particulate (DEP) and the oxidative stress status. For that purpose, a survey has been conducted in real occupational situations where workers were exposed to DEP (bus depots).

Several exposure parameters have been considered: particle concentration and size distribution, elemental and organic carbon, particulate heavy metal content (Fe, Cu, Mn), surface functional groups present on particles, NO_x and O_3 concentrations. A biomarker of oxidative stress (8-hydroxy-2'-deoxyguanosine [8OHdG]) and the level of antioxidants have been quantified in urine of volunteers.

Results indicated that the occupational exposure to particles was rather low (40-80 □g/m^3 for PM_4). Urinary levels of 8OHdG increased significantly ($p<0.05$) during two consecutive days of exposure for non-smoking workers. This increase of oxidative stress was accompanied by an increase of urinary levels of antioxidants, confirming the defence mechanism proposed in [1]. A statistical model, obtained by multiple regression analysis, showed that 45% of the increase of 8OHdG could be explained by organic carbon, surface area of $PM_{0.7}$ and NO_2/NO_x variables.

[1] A. Nel et al, 2006. Science, 311, 622-627.

Use of a battery of cytotoxicity and genotoxicity testing methods to assess TiO_2 and SiC nanoparticles toxicity *in vitro*

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The development of nanotechnologies may lead to considerable release of nanomaterials in the environment, that can be potentially toxic for human health and environment. Inhaled nanomaterials might cause damage to respiratory tract, particularly to the alveolar compartment. In this context, our research is focused on the response of eukaryotic cells to nanomaterial exposure.

We used *in vitro* cell systems to model lung exposure (A549 alveolar epithelial cells). A549 were exposed to five different TiO2 nanoparticles which were previously shown to be internalised in these cells, as well as to six different SiC nanoparticles. Cytotoxicity was assessed by MTT and LDH, and the alkaline comet assay, gamma-H2AX immunostaining and micronucleus tests were used to evaluate nanomaterials genotoxic effects.

Our data demonstrate that nanoparticles toxicity depends on their size, morphology and chemical composition. The smallest nanoparticles (diameter 10-25 nm) and the spherical ones induced toxic effects whereas particles with diameter superior to 100 nm and elongated ones did not. Cell death only reached 25-30% of cell population after 48 h of exposure to 50-200 µg/ml nanoparticles. Genotoxic effects were detected *via* the alkaline comet assay after 24 and 48 h of exposure of A549 to TiO_2 nanoparticles, but neither micronuclei nor double strand breaks were induced. The originality of this study lies on the panel of well characterized nanomaterials which were tested on the same cell line with several methods. These data lead to a better understanding of nanomaterial cytoxicity and a better learning about the undergoing mechanism of genotoxicity.

Development and characterization of a quantitative and easy-to-use system for thin-film deposition of nanoparticle suspensions onto cells

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A soft-mist-exposure unit was developed for controlled thin-film deposition of solutions or nanoparticle suspensions onto cells at the air-liquid interface (ALI). Up to 5 ml of suspension is nebulized by a vibrating membrane droplet generator and homogeneously distributed throughout an exposure chamber where the droplets efficiently deposit (~55%) due to gravitational settling onto two cell culture plates transwell inserts containing the cells. If desired, (e.g. for mimicking exposure conditions in the lungs) the chamber can be conditioned to 37C and about 95% relative humidity. For 1 ml of suspension the exposure is completed within 10 min and a uniform film of about 10 µm is formed on the cells. Exposures can be repeated with a low degree of variability (<15%) and the transwell inserts can easily be removed and processed for measuring cell response parameters. Initial tests with A549 lung epithelial cells have shown no impairment of cell viability during the exposure process, but significant differences in cellular mRNA/protein response (IL-8, HO-1) were observed when comparing ZnO particle exposures at the ALI and under submerged conditions.

Cytotoxicity studies of photocatalytic titanium dioxide nanoparticles in the presence of UV-light

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The cytotoxic effects of five different titanium dioxide nanoparticles on various mammalian cell lines have been examined. The particles differed in their size, BET-surface area and in their optical properties. In order to cover different possibilities of absorption into the human body, fibroblast cells (NIH-3T3), lung carcinoma cells (A-549), liver carcinoma cells (HEP-G2) and adrenal pheochromocytoma cells (PC-12) were tested.

Due to their properties as a semiconductor, titanium dioxide nanoparticles may form reactive radicals when exposed to ultraviolet radiation. These radicals are a potential hazard and may be part of the cytotoxic mechanism. For this reason, the influence of the photocatalytic activity of the nanoparticles on their cytotoxicity has also been studied. During the cultivation with nanoparticles, the cells have been irradiated with different doses of UVA-light (5-20 $kJ \cdot m^{-2}$). To evaluate the cytotoxicity, the viability of the cells has been determined using the MTT-assay.

The results show no significant change in viability while cultivating the cells with low concentrations (< 500 $\mu g \cdot mL^{-1}$) of nanoparticles. However, a decrease in viability was observed in some of the experiments with high concentrations (≥ 500 $\mu g \cdot mL^{-1}$) of nanoparticles. Furthermore, the experiments conducted with UVA-irradiation did not show a significant increase in cytotoxicity.

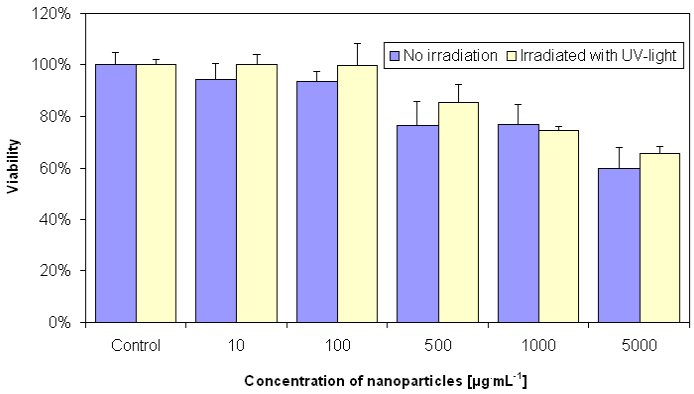

Figure 1: Viability of A-549 Cells cultivated with Kronos vlp 7000 particles with and without UV-irradiation (20 kJ·m-2 UV dose).

Nanoparticle Synthesis & Characterisation: Understanding Role of Physiochemical Properties in Bio-Nano Interactions.

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Nanotechnology is expected to be the basis of the main technological innovations of the 21st century, including curing disease (nanomedicine), earlier diagnosis of disease (nanodiagnostics) and faster electronics (nanotechnology). An estimated 30,000 different nanomaterials are being developed worldwide, including semiconductor quantum dots, carbon nanotubes, and metal nanowires. Responsible development of nanotechnology requires that in addition to optimising the applications of nanotechnology, a complete assessment of the potential risks to human health and to the environment associated with the production, use and disposal of nanomaterials is undertaken in parallel.

Due to their small size and their large surface area nanoparticles have different properties that larger pieces of the same material do not possess. Their very tiny size means that they can enter into individual cells easily and once inside they may trigger unknown cellular processes. The general problem to be addressed here is that we seek a correlation between the nanoparticles characteristics (size, shape, surface characteristics) and the uptake by, transport around and final intra-cellular location of the nanoparticles into cells. Nanoparticles-cells interactions are studied in the presence of a solution of proteins, thus nanoparticles in physiological conditions invariably adsorb proteins on their surface. There results a disrupted outer surface layer of protein (adsorbed to and induced by) the particles that is in direct contact with the cells.(1, 2) It is this outer protein layer that is in direct contact with the cell surface and thereby provides for the main nanomedical and nanotoxicological effects. This is a central feature of our work.

The aim of this project has been to synthesize and completely characterize a series of "smart" copolymer nanoparticles increasing charge and hydrophobicity for use in nanomedicine. Nanoparticle surface properties are varied systematically, allowing investigation of the impact of surface charge on observed responses in cells. Addition of a covalently-bound fluorescent label to the particles ensures that we track the uptake and sub-cellular localisation of the nanoparticles. Key issues to ensure quantitative results are to ensure that the nanoparticles are properly dispersed in the relevant test media in order to control the dose of nanoparticles exposed to cells, and that no free dye remains in the particles that can elute out and produce false uptake kinetics. A range of visualization techniques, such as microscopy using coloured labels, have been used to follow the progress of the nanoparticles inside/outside cells. Preliminary results from interaction studies with cells are presented.

(1) *Lynch I, Dawson KA, Linse S. Detecting cryptic epitopes in proteins adsorbed onto nanoparticles, Sci. STKE, 2006, 327, pp pe 14.*

(2) *Cedervall T, Lynch I, Lindman S, Berggård T, Thulin E, Nilsson, H, Linse S, Dawson KA. Understanding the nanoparticle protein corona using methods to quantify exchange rates and affinities of proteins for nanoparticles, PNAS, 2007, 104, 2050-2055.*

NanEAU - Toxicological effects of emerging nanoparticles in water on aquatic models organisms and uptake in humans from drinking water – a project description

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Manufactured nanoparticles (NPs) show great technical promise, but little is known about their effects on living systems. NPs exhibit special physico-chemical properties and reactivities due to their small size and homogenous composition, which are not present at the larger scale. Depending on their use, NPs will potentially pollute subsurface aquifers, surface lakes and rivers. Recently, critical questions have been raised regarding the impact of manufactured NPs on the environment and human health.

NanEAU is a 2-year project funded by the Fonds National de la Recherche in Luxembourg. The aim of NanEAU is to adapt and develop test protocols for the evaluation of (environmental) toxicity of emerging NPs. In addition to classical assays using established biomarkers and standardized procedures, novel-predicting biomarkers applying proteomic and genomic approaches will be developed. The possibility of uptake from the water in higher vertebrates and intracellular localization will be studied.

NPs will be selected in close cooperation with the ongoing FP7 project NANOTEST to make best use of the expertise available in this project. The experiments as proposed in NanEAU will allow a better description of hazards and risks associated with the uncontrolled release of NPs into the environment and will contribute to a sound scientific and holistic understanding of toxicological aspects as effects will be studied from a protein to the organism levels and from algae to complex vertebrates.

The expected outcome of NanEAU is additional information on behaviour and interaction of NPs in the water, its potential risks for a range of organisms, and new biomarkers for the presence and effects of NPs supporting the sustainable use of water resources.

Impact of Nanomaterials on Human Health: Lessons from in vitro and animal models

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The rapid expansion of nanotechnologies promises to have significant benefits to society, yet there is increasing concern that exposure to engineered nanoparticles (ENPs - structures with a size of ≤ 100nm in at least one dimension) will have negative impact on human and environmental health. In spite of the recent advances it remains unclear how ENPs interact with their targets and which of the particle metrics are driving or modulating the responses. The goal of this project is to understand biological effects induced by ENPs, at different levels of biological organization (cells, tissue, organisms), from which the potential risks to human health can be estimated in a hazard and risk assessment framework. The mechanism of action of ENPs will be studied with focus on oxidative stress, inflammation, genotoxicity and reproductive toxicity. We will characterize selected ENPs (TiO_2, Ag); determine changes in their physico-chemical properties following interactions with environmental factors; determine intrinsic toxicity on molecular/cellular levels; study direct and expressed toxicity (acute vs chronic), tissue-specific effects, excretion and DNA repair capabilities. Specific toxicity includes apoptosis/necrosis, genotoxicity (comet assay, histone γH2AX foci, DNA base damage, micronucleus test), oxidative stress and its impact on antioxidant cellular defence, changes in gene expression, especially cellular signalling pathways. The evaluation of reproductive toxicity in representative mammalian models will give information on potential long-term toxic effects to humans, as well as the consequences at the population/community level. Our research will contribute to the development of appropriate risk assessment methodologies that can be used in an effective way for wealth creation, assisting regulators and environmental managers to protect human health.

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Nanoparticle internalisation and cellular effects in endothelial and glioblastoma cells : evaluation of drug delivery in cancer therapy

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New drug delivery technologies must be designed to surmount biochemical and anatomical barriers and safely allow transport of biomolecules and protein-based drugs to specific intracellular targets. Due to their very low size, nanoparticles have become very attractive. Carbon-based nanomaterials present particular interest, since they are chemically inert, and can be surface functionalised for the grafting of nucleic acids and proteins. This way may allow to specifically target cell compartments and hence lower drug concentration, reducing side effects. For all these reasons nanodiamond-based therapy may significantly improve cancer treatment.

Aim of the European project “Nano4drugs” was to develop peptide-grafted diamond nanoparticles, including nanodiamonds containing nitrogen-vacancy fluorescent colour centres (NV), allowing single particle tracking into the cells. We have studied the uptake and the cellular effects of two kinds of nanodiamonds in endothelial and glioblastoma cells: irradiated fluorescent nanodiamonds (HPF2) and colloidal, functionalised detonation nanodiamonds (OND75). Dose-response (20-100 µg/ml) and time course experiments were performed. Both types of nanodiamonds were efficiently internalized, as shown by optical, fluorescence and transmission electron microscopy and by flow cytometry. Internalized nanodiamonds did not produce significant cytotoxic effects (MTT assay), at doses lower than 100µg/ml, and did not affect microtubular cytoskeleton and cell morphology. In particular, transmission electron microscopy showed that nanodiamonds were internalized by endothelial cells at higher extent than glioblastoma U87-MG cells. Internalized nanodiamonds accumulated in specific intracellular compartments. Further experiments are going to identify these compartments and to better characterize the specific route of nanoparticles.

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DTT consumption by a panel of carbonaceous and metallic nanoparticles as a measure of their oxidative potential

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The knowledge of physicochemical properties of nanoparticles (NP) is relevant due to the key relationship between physicochemical structure and toxicity. Therefore characterisation of surface reactivity is of prime importance. The oxidative properties of a panel of various NP (*Carbonaceous*: Printex 90, Diesel EPA, Nanotubes, Fullerene; *Metal oxides*: Al 7 nm, Al 50 nm, Al 300 nm, Ce, Ni, Si, Zn, Ti (anatase), Ti (rutile), and *Metal*: Ag) were studied with an acellular reactivity test measuring dithiothreitol (DTT) consumption (Sauvain, Deslarzes, and Riediker 2008).

NP were diluted and sonicated in Tween 80® to a final concentration of 50 □g/mL. Only Printex 90 needed to be diluted 5 times before doing the DTT assay because of its most probably high activity. Suspensions were characterised for NP size distribution by nanoparticle tracking analysis (Nanosight©). Fresh solutions were incubated with DTT (100 µM). Aliquots were taken every 5 min and the remaining DTT was determined by reacting it with DTNB. The reaction rate could thus be determined for NP suspension and blank in parallel.

All the carbonaceous NP appeared to be able to catalyse the oxidation of DTT by O_2. Printex 90 was the most reactive, followed by Diesel EPA and then Nanotubes. Fullerene was practically non-reactive. The DTT test was non-responding for most of the metallic NP, except for Nickel oxide and Ag that presented a significant positive reactivity. In contrast, Zinc oxide showed a significant negative value, suggesting “stabilization” between ZnO and DTT, and Zn^{2+} complexation with DTT.

In the long run an understanding of the relationship between nanoparticle physicochemistry and toxicity could allow a predictive structure/activity model. Studies such as the present one makes a contribution to such a structure activity model of NP toxicity. Characterisation of structural physicochemistry also needs to be specifically addressed in the media used in toxicological testing.

Sauvain JJ, Deslarzes S, and Riediker M. 2008. Nanoparticle reactivity toward dithiothreitol. Nanotoxicology 2 (3): 121-12.

The role of particle uptake for cytokine responses induced by amorphous silica nanoparticles in epithelial lung cells

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Nanoparticles (NPs) of amorphous silica are used in a large range of products. Inhalation of such NPs may induce inflammation, and represent a health hazard. In this study we have examined the relationship between cellular uptake of the silica NPs (50 nm) and cytokine expression and release in a human bronchial epithelial cell culture (BEAS-2B). Rhodamine-labelled NPs were used, and the cellular uptake was studied by confocal microscopy. The cytokine responses (IL-6, IL-8, IL-1, TNFalpha) were examined by ELISA and by real-time PCR. Both uptake and cytokine responses were determined at different culture conditions giving different extent of particle agglomeration. The silica particles induced a rapid (1-2 h) and strong mRNA expression of all the cytokines. At his time point only a small amount of particles were taken up by the cells. The results indicate that particle uptake is not required for eliciting cytokine responses by rhodamine-labelled silica NPs, which suggests that these responses are initiated at the cell surface.

Assessing nanoparticle exposure in powder handling and use of nanofilm spray products

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Suitable methods for prediction of risk of exposure to nanoparticles (NPs) prior to e.g., full-scale use, is highly warranted. Handling nanoparticle powders or working with processes generating NPs are probably the operations with the highest risk of exposure today.

Usually, the first questions raised are: 1) What is the level of exposure? and 2) What is the size distribution of the particles? However, NP risk assessment may be improved significantly using combined measurements of mass-, volume-, surface-, and number size distributions and if possible combine these measured with data on chemical reactivities of the NPs, such radical formation capacity or redox activity, and biodurability.

These strategies are discussed based on results obtained for assessing the characteristics and risk of exposure to airborne NP's generated during powder handling and use of nanofilm sprays: 1) single-drop and rotation drum dustiness testing using a downscaled EN15051 rotating dustiness drum equipped with filter sampling of inhalable dust and on-line particle sizing [1,2]; and 2) source strength analysis of particles during application of spray-based nanofilm coatings in a stainless steel chamber and on-line particle sizing [3]. In both methods, the particle concentrations and size distributions were measured every second using a TSI Fast Mobility Particle Sizer (Model 3091) and a TSI Aerosol Particle Sizer (Model 3321) for measuring particles between 5.6 nm and 20 µm.

The tests have shown great differences in particle size distributions, exposure levels (source strength) and thereby exposure risk depending on type of material as well as handling methods and particle generation process. The data are further discussed with respect to deposition and chemical reactivity.

Schneider and Jensen Ann Occup Hyg (2008) 52/1, 23–34 Jensen et al. J Nanopart Res (2009) 11:133–146 Nøjgaard et al. Env Sci Techn (submitted)

Respiratory protection factor in atmosphere contaminated with CBR nanoparticles

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Personal protection from exposure to nanoparticles is important due to the expansion of nanotechnology and the necessity of adequate personal protection against bioaerosols and primary viruses that appear dominantly in the nanodiameter range. In this paper, the factional deposition of particles in respiratory systems is described as well as the role of particle number and surface area on health impact, in the aim of re-examining current and developing future appropriate methodology for testing personal protective equipment against contamination by nanoparticles. Total inward leakage (TIL) of any respirator represents the sum of leakage through the filter, then trough the facial seal, and at the end through the exhalation valve as well as any other pathways. Also, a standard method for inward leakage measurement was discussed and a frame for developing a method for fractional inward leakage and fractional protection factor assessment was discussed. When considering respiratory protection against nanoparticles, the problem of seal leakage is more uncertain around the face than through the filter. Distributions of protection factor tested for thirty persons (for each person repeated once) and for two persons, one female and one male (for both persons repeated thirty times), are shown. Protection factor was tested for one half mask and one full face mask, with NaCl aerosol, “Moore’s” Test Rig CEN Bench Rig, (MMD=0.47 μm, σg = 2.21, CMD= 0.071 μm) and ambient aerosol measured with TSI particle number counter.

Maturation response of human CD34$^+$ progenitor-derived dendritic cells exposed to engineered nanoparticles

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To study the potential immune-stimulating effect of engineered nanoparticles (NP), human immature CD34$^+$ progenitor-derived dendritic cells (CD34-DC) were exposed to spherical gold (4.3 and 13 nm), iron oxide (6 nm) or cobalt (4 nm) NP for 24 and 48 hours. Mono-dispersed NP in solution and freshly resuspended nanopowders were used at the same concentration per type of NP. The maturation response of CD34-DC was measured by determination of cell surface expression of the DC surface markers HLA-DR, CD86, CD83 and CD54 using flow cytometry. Additionally, potential inhibition of cellular growth by the NP was analysed by means of the alamarBlue™ and WST-1 assays. Microscopically, freshly resuspended nanopowders were observed to be more likely to form aggregates, compared with NP in dispersion. No reduction of CD34-DC growth was observed in response to all NP tested at different concentrations. At the highest concentration tested, ranging from $2.4*10^{11}$ to $2.0*10^{13}$ NP per ml for the different particle types, neither the mono-dispersed NP, nor the freshly resuspended NP could induce maturation of CD34-DC after 24 and 48 hours of exposure, i.e. no significantly increased expression of the DC maturation markers HLA-DR, CD86, CD83 or CD54 was measured when compared to control conditions. When the cells were co-treated with the cytokines tumour necrosis factor-α and interleukin-1β (5 ng/ml), which are known to mature CD34-DC, none of the mono-dispersed NP was able to further enhance the maturation response. Small spherical, engineered NP were found not to be cytotoxic to CD34-DC when added either as mono-dispersed NP solution or freshly resuspended nanopowders. Furthermore, they were not able to potently trigger DC maturation. Comparison of our data with other toxicological endpoints and different NP (size, composition, surface coating, shape, …) is warranted. Future work may also include the use of transcriptomics to reveal potential changes at multiple sites in cells.

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In vitro mammalian cytotoxicological study of PAMAM dendrimers –towards quantitative structure activity relationships

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Dendritic polymer nano-particles are highly branched radial polymers that have specific and systematically variable size, shape and chemical structure. Polyamidoamine (PAMAM) dendrimers are one such dendrimer having a mono-disperse, stable and porous molecular architecture which is very effective for drug formulation and many other biomedical applications, e.g. MRI contrast agent, DNA transfection, in siRNA mediated gene silencing etc. Its radial structure contains a 2-carbon ethylenediamine core and primary amino groups on the surface. Successive generations (referred to as G0-G10) have increasing diameter and double the surface functional amino groups per generation. This present investigation focused on the *in vitro* toxicological assessment of full generation PAMAM dendrimers of generation 4, 5 and 6.

Two mammalian cell lines, HaCaT, an immortal non-cancerous human keratinocyte cell line and SW480, a primary adenocarcinoma cell line of the colon were employed for cytotoxicity testing. Alamar Blue (AB), Neutral red (NR) and MTT assays were used for short term (24 h) and clonogenic assay for long term (8 days) toxicity testing. The diameters and zeta potentials of each generation of dendrimers were measured.

Results showed that the cytotoxicity of PMAM dendrimers increased with increasing generation and therefore increasing diameter and surface amino groups. PAMAM G4 was the least toxic, followed by G5, and G6 was the most toxic among them. Following short term exposure, the MTT assay appeared more sensitive than AB and NR assays, where NR assay was least sensitive. Overall, the clonogenic assay showed the highest sensitivity with EC_{50}'s of PAMAM G 4, 5 and 6 in HaCaT cells ranging between 2.6, 0.6 and 0.2 μM, respectively and in SW480 cells between 1.4, 0.4 and 0.2 μM, respectively. The systematic variation of toxic response is well correlated with particle surface area and zeta potential, pointing towards structure activity relationships.

Pneumotoxicity of silver coated nanocomposites. An experimental study on rats.

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The antibacterial effect of silver nanoparticles has resulted in their extensive application in health, electronic, and home products. Although a number of studies on silver dust, and silver compounds have revealed some insights, little is yet known about the toxicity of nano-sized silver particles. Thus, the lung toxicity of silver nanoparticles is of particular concern to ensure the health of workers and users.

Two sorts of nanocomposites have been used: titanium dioxide coated by silver ions (TiO_2-Ag) and zirconium phosphate-based ceramic ion-exchange resin containing silver nanoparticles (Res-Ag). The mean diameter of tested nanocomposites was lower then 300 nm. Adult male Wistar rats were exposed to intratracheal single dose of 2.0, 5.0 and 10.0 mg nanocomposite in 0.3 ml saline. After 24, 72 hr, and at 1, 4, 13, 26, 40 weeks bronchioalveolar lavage (BAL) was collected for biochemical testing, while lungs, lymphatic tissue and other inner organs were collected for histopathological evaluation.

After 24 – 72 hrs an acute, non specific inflammatory response was observed in lugs of all animals. Total protein, LDL activity and Clara cell protein (CC16) were significantly elevated. The particles were seen in cytoplasm of macrophages without signs of cytolysis. The degree of inflammation was dose-dependent and decreased during next weeks of observation. An increased thickness of alveolar septa and small cellular granulomas without progressive fibrosis were noted in 26 – 40 week of observation. Res-Ag induced greater degree of changes then TiO_2-Ag. Translocation of tested materials to the peribronchial and mediastinal lymph nodes was noted after 24 hr observation period. Since 2 week particles could be noted in the in the spleen and liver.

Tested silver coated nanocomposites, which to used as bactericide for textiles revealed non specific response in respiratory tract without progressive fibrosis. Particles may be translocated from lungs to other organs.

Analysis of interactions and potential cytotoxicity of industrial nanoparticles and thin films on the murine macrophages RAW 264.7 cell line

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Few studies that have been reported suggest that ultrafine (P-25) TiO2 particles produce greater pulmonary inflammation when compared with fine-sized TiO2 particles. However, in contrast to the conclusions of the earlier studies with P-25 type ultrafine TiO2 particles, the results of recent preliminary studies comparing the effects of nano- vs. fine-sized particles, have indicated that pulmonary exposures in rats to uncoated TiO2 nanoscale rods (200 nm lengths × 30 nm diameters) and TiO2 nanoscale dots (particle size < 30 nm) did not produce enhanced lung inflammation in rats when compared to fine-sized TiO2 particle exposures (particle size ~ 270 nm). In this work thin films (TiO_2, TiO2:In, TiO_2:WO_3, TiO2:Fe2O3) and nanoparticles (TiO_2, TiO2:In, TiO_2:WO_3, TiO2:Fe2O3, CeO, $ZnPO_4$) were prepared and characterized by SEM, XRD, AFM. Cytotoxicity analysis by Trypan blue method and MTS assay, morfological analysis by SEM and mTNFα ELISA determination were performed on cells incubated on thin films and particles (25µg/ml). Cell proliferation and mitochondrial activity data don't show average reduction compared with the control after 12, 24 and 48 hours of incubation on thin films and with particles. SEM analysis confirmed the presence of agglomerate of 100-150 nm particles that interact with cells. The degree to which engineered nanoparticles aggregate in vitro or in aerosol or in occupational environment and subsequently do or do not disaggregate following inhalation and particle deposition in the lung should strongly influence particle deposition rates as well as interactions. We also present data showing macrophages activation connected to the contact with nanoparticles. TNF-α release of RAW 264.7 cells have been determined after 5 and 24 h of incubation with nanoparticles (25µg/ml). TNF-α production is higher after 5 hours of treatment, in particular with TiO_2 and TiO_2-In nanoparticles. Average TNF-α release decrease after 24 hours of treatment except for TiO_2-In , CeO_2 and $ZnPO_4$ nanoparticles confirming their potential cytotoxicity.

In vitro assays for evaluation of nanomaterials cytotoxicity mechanisms

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Engineered nanomaterials are increasingly being used in medicine for in-vitro diagnostics, imaging and for drug/vaccine delivery. The properties of nanomaterials may differ substantially from the corresponding bulk materials. Undesirable properties may result in harmful interactions with biological systems. For this reason, the establishment of principles and test procedures that ensure safe use of nanomaterials is urgently required. Currently, standard test procedures that are uniformly applicable to all nanomaterials do not exist, due to the large physicochemical variability of nanomaterials (i.e., surface area, size, chemical composition, solubility, shape, aggregation). Nevertheless, nanomaterial toxicology is a rapidly growing field, as is reflected by the rapid increase in publications in the last few years. In the context of the EU FP6 project NACBO (Novel and Improved Nanomaterials, Chemistries and Apparatus for Nano-Biotechnology) we tested the *in vitro* toxicity of several nanomaterials developed for optical imaging purposes. These agents contain, based on the synthesis route chosen, lanthanides complexed with the chelating agent EDTA. The toxicity investigation was carried out in three human-derived cell lines (U-937, K-562 and MCF-7), selected on the basis of representative phenotype. A flow chart for the analysis of nanomaterial toxicities has been previously established (1) and includes a cell proliferation assay (MTS assay), a live/dead viability test (LDH release), a mitochondrial toxicity evaluation (JC-1 assay), apoptosis/necrosis (PI), and gene expression studies (microarray analysis). Comparative analysis of the toxicities of the nanomaterials and their separated components show that the toxicity found in two out of three cell lines is mainly caused by the presence of EDTA in the nanoparticles suspension.

1. Galluzzi L. et al. In vitro evaluation of nanomaterials toxicity. ESF-EMBO Symposium. Sant Feliu de Guixols (Spain) Nov 3-8, 2007

Toxicity of Copper based nanoparticles on earthworms

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Nanotechnology is playing an increasing role in society. The use and release of nanomaterials e.g. quantum dots will result in nano-sized contaminants in the environment and the biota. Although nano-sized particles (NP) are already present in nature, the novel man-made specific designed molecules entering the environment may cause toxicity in organisms not previously exposed to nanoparticles, or at least not this kind of particles.

This presentation deals with the toxicity of Copper based nanoparticles (Cu-NP) to soil dwelling organisms. The aim was to evaluate their lethal and sub-lethal toxicity to the earthworm Eisenia fetida.

The results show that the most sensitive toxicological parameter in the present study was reproduction (cocoon production) and avoidance, with hatchability, growth and mortality being affected only at higher concentrations. The Cu-NPs exposure displayed a dose-response relationship.

Risk Assessment As A Mean To Address The Potential Risk Of Nanomaterials - Too Little, Too Late?

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Risk assessments are often necessary to support regulations and have repeatedly been proposed as means to inform decision-makers about the risks of nanomaterials (NM). The question is whether it is adequate to ensure a decision-making process that enables us to make informed decisions within a reasonable period of time, despite large uncertainties about the risks of NM. In this study the feasibility of applying risk assessment to NM is investigated within each of the four steps of chemical risk assessment i.e. hazard identification, dose-response assessment, exposure assessment, and risk characterization. It was found that each step holds a number of limitations and flaws when applied to NM. For instance, it is not yet possible to systematically link reported nanoparticle properties to the observed effects for effective hazard identification. For dose-response assessment, it was unclear whether a no effect threshold can be established and what the best hazard descriptor(s) of nanoparticles is and what the most relevant endpoints are. Exposure assessment is hampered by difficulties in monitoring NM exposure in the workplace and environment due to unknown exposure pathways and the paucity of knowledge and lack of access to information. In risk characterization, the sum or maybe even the power of all these limitations are conveyed to calculating risk quotients for nanoparticles. Considerable work is still required if future risk assessment of current NM and products is to be relevant and reliable. There are a number of additional problems when it comes to risk assessment including the lack of standards, the diversity of NM and their applications which hampers case-by-case risk assessment especially when we consider the pace of the technological development. It is concluded that risk assessment is inadequate to timely inform policy-makers about the health and environmental risks of NM, if not in the short term, then most definitely, in the long term.

Application of organotypical slice cultures for investigating selective neurotoxicity of nanoparticles

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Nanomaterials have unique properties and applications, especially within diagnostics and drug delivery in medicine. However, the properties that make nanoparticles so useful could also be coupled to unintentional health effects. The main characteristic of nanoparticles is their size. As the size decreases the relative surface area increases, as does the reactivity, and nanoparticles can thus generate distinct effect not seen with the same material in a larger form. Nanoparticles can pass cell membranes, facilitating uptake into blood or lymph circulation, and also get into tissues normally protected by barriers, such as the brain. The fact that nanoparticles can get access to the brain via olfactory neurons or by crossing the blood brain barrier raises the possibility that nanoparticles can interfere with brain function. Inhaled nanoparticles have been detected in the olfactory bulb and also in deeper brain regions, as the hippocampus. So far there is scant knowledge about effects of nanoparticles in the brain. Toxicity can be selective to glial cells or neurons, or to specific cell populations.

Selective toxicity in the brain can be studied *in vitro* in organotypical hippocampal slice cultures. The mechanisms underlying selective vulnerability is important to obtain a better understanding of neurodegeneration in general. Cell death analysis suitable for large scale experiments can be performed by use of propidium iodide. Fluorescence analysis can be performed on living cultures, and allows repeated measurements in the same culture at different time points after exposure. The slice cultures can then be applied for e.g. immunocytochemistry for investigations of underlying mechanisms of toxicity. Slice cultures can also be made from other brain regions, e.g striatum which is relevant for Parkinson´s disease. We will apply slice culture models for testing of neurotoxicity of medical nanoparticles and underlying mechanisms of toxicity (*Supported by EC NanoTEST201335).*

C_{60} fullerene a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays

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Since their discovery in 1985 fullerenes, nanospheres of carbon have attracted attention regarding their physico/chemical properties. The production of fullerenes has reached an industrial scale without knowing about the environmental and human health impact. The toxicity of C_{60} is controversially discussed.

In aim of this study was the comparison of well characterized tetrahydrofuran (THF) suspended C_{60} with water-stirred C_{60} suspension and their effects on crustacean *Daphnia magna* an indicator for ecotoxicolocigal effect (according to OECD 202) and human lung epithelial cell line A549 as a simplified airway wall model.

In the present study in THF suspended C_{60} suspension a water soluble THF-peroxide compound was formed which was mainly responsible for the cytotoxic effects and probably not related to C_{60}. The THF suspended C_{60} suspension, additionally washed, induced a slight oxidative effect measured by the presence of free oxygen radicals; whereas water suspended fullerenes was acting as a mild radical scavenger. No sever effects of C_{60} were observed neither in A549 cells *in vitro* nor in *Daphnia magna*.

This study underlines the need for in-depth material characterization prior to any toxicological evaluation.

Reference:
P.Spohn, C. Hirsch, F. Hasler, A. Bruinink, H.F. Krug, P. Wick (2008) C_{60} fullerene a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays, Environ. Pollut. doi:10.1016/j.envpol.2008.08.013

Pro-inflammatory responses induced by carbon black and titanium dioxide nanoparticles in bronchial epithelial cells: need of multiparametric evaluation due to adsorption artifacts

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The initiation of an inflammatory process is the main adverse effect observed following the exposure of the airway epithelium to nanoparticles (NPs). This study was designed to explore the pro-inflammatory potential of two different NPs of similar size but of different chemical nature (CB 13nm and TiO_2 15nm) on a human bronchial epithelial cell line (16HBE14o-).

This pro-inflammatory response was evaluated in terms of release as well as intracellular production and mRNA expression of three different pro-inflammatory cytokines: IL-6 (Interleukin 6), GM-CSF (Granulocyte Macrophage Colony Stimulating Factor) and TNFα (Tumor Necrosis Factor alpha). Exposure to NPs induces a dose-dependent expression of mRNA of all these cytokines depending upon the chemical composition of NPs. The detection of released cytokines by ELISA appeared to be an inaccurate methodology to evaluate the pro-inflammatory response. Indeed, we observed that NPs adsorb cytokines and that this binding is dependent on the nature of both, the cytokine and the NPs. Furthermore, addition of foetal calf serum or bovine serum albumin improves the detection of cytokines but also reduces cellular responses. Use of different detergents (Tween, Triton and NP40) demonstrated limited efficiency to desorb cytokines from NPs. Furthermore, we studied the role of oxidative stress in this pro-inflammatory response. Indeed, the evaluation of intracellular hydroethidine oxidation showed that CB and TiO2 NPs both induce a dose dependant increase of oxidative stress. Use of catalase allows to diminish the oxidative stress induced by these NPs as well as to decrease the pro-inflammatory response.

To conclude, this study demonstrated the pro-inflammatory potential for CB and TiO_2 NP but underlines the methodological artifacts faced during the *in vitro* evaluation of cytokine release that necessitates a multiparametric evaluation. This pro-inflammatory response is due to the oxidative stress induced by these NPs

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Characterizing exposure to laser generated nanoparticles

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The laser is a versatile tool for a large variety of production processes, e.g. cutting, welding and surface treatment. While working with laser, the primary risk is exposure to radiation, the secondary risk consists of exposure to particulate or gaseous emissions.
A few studies have been conducted measuring particle emission during laser machining. These results show that most particles are in the range of 0,03 µm to 0,5 µm. Not much attention have yet been paid in characterizing this submicron fraction.

The aim of this study was to gain insight in the characteristics of emitted nanoparticles during the operation of four different lasers (Nd-Yag, Diode, CO2, diode pumped Nd:Yag with hybrid welding head) conducting different activities: welding, cutting and surface treatment.

Real-time measurements of nanoparticle number concentration, particle size distribution and lung deposited surface area were conducted. Off-line analysis of electron microscope samples provided information on particle morphology. The chemical composition of some nanoparticles was determined using Energy Dispersive X-ray spectroscopy.

The maximum number concentration was observed for Nd-Yag cutting, with 8,25 E+06 particles cm-3. Each of the above mentioned lasers, induced the formation of different concentration ranges and size distributions of nanoparticles.

Also, similar successive laser activities with the same type of laser resulted in different size distributions. For example, for CO2 laser welding a shift in size distributions from wide distributions with smaller particles to narrow size distributions with bigger particles was noticed. For Nd-Yag diode welding (hybrid) variations in size distributions were seen due to addition or no addition of metal powder.

The detailed analysis of microscopic images showed that laser welding and cutting created mainly particles with a soot-like morphology consisting of Fe, Cu, Si and Zn. SEM-images retrieved from cladding resulted in more discrete particles consisting of Cu and Fe.

First results showed that number concentrations, size distributions, lung deposited surface area and microscopic images differ in each situation significantly caused by the differences in lasers, in laser activities, in metal powder addition and in local exhaust ventilation systems.

The Evaluation Of The Cytotoxic Effects Of Nano Particulate Silver and Zinc Oxide On An Intestinal Model.

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Nano ZnO and Ag have been shown to display antimicrobial effects this has lead to their application in a number of areas such as antimicrobial surface coatings, anti bacterial wound dressings and more recently in polymer composite systems for use in food contact materials or so called “smart packaging”. Concerns have been raised due to the incorporation of nanoparticles in food packaging stemming from the possibility of repeated low dose direct exposure, through ingestion, primarily due to degradation and nanoparticle leaching from the polymer composite. In this preliminary study two human colorectal carcinoma cell lines, namely. HT29 (ATCC No : HTB-38) and SW480 (ATTC No : CCL-228) were employed as an intestinal model. Nano ZnO and Ag were employed as test particles prior to any cellular studies a full particle size characterisation was carried out by the following techniques Dynamic Light Scattering, TEM and AFM. The cytotoxic potential of nano scale ZnO and Ag was then evaluated using 4 cytotoxic endpoints namely the Neutral Red, Alamar Blue, MTT and Clonogenic assays. Direct exposure of both cell lines to the nanoparticles revealed an effect which was dependant on particle, received dose and time. The results of these studies and their implications for nanoparticle composite systems in the food sector will be discussed.

Dietary Toxicity of Single-Walled Carbon Nanotubes and Fullerenes (C_{60}) in Rainbow Trout (*Oncorhynchus mykiss*): Histopathology and Oxidative Stress

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The development and manufacture of carbon nanoparticles (CNPs) has increased rapidly, and as these nanoparticles become incorporated in more consumer products there is a risk of environmental contamination. This is a concern because some studies indicate CNPs as potential respiratory toxicants and/or inducers of oxidative stress. The objective of this investigation was to determine the effects of single-walled carbon nanotubes (SWCNTs) and carbon fullerenes (C_{60}) in a chronic dietary exposure in rainbow trout. Feeds were prepared by spraying aqueous emulsions of CNPs (CNPs dispersed with sodium dodecyl sulphate and sonication) on rainbow trout feed pellets. Juvenile rainbow trout were fed 2.8 % of their body weight daily with either a control, 500 mg SWCNT kg^{-1}, or 500 mg C_{60} kg^{-1} for six weeks, followed by a two-week recovery period. Fish were sampled during the exposure period to determine growth, and histological and biochemical lesions. Growth of fish did not differ among treatments (SGRs between 1.84 and 1.96 %) or compared to controls (ANOVA $P > 0.05$) following the 6-week dietary exposure, or during the additional 2-week recovery period. Exposure of fish to CNPs did not affect growth or haematological parameters. Indications of oxidative injury (elevation of TBARS; induction of total glutathione) were detected at discreet time points in fish exposed to SWCNTs; however, results at later time points suggested recovery from oxidative stress. Lesions were detected by histopathology in 2 of 6 livers examined after C60 exposure; however, there were no acute lesions in the gill, intestine, or brain after exposure to CNPs. The results of this study suggest some hazards associated with food route exposures, with implications for food safety.

Dietary Toxicity of Single-Walled Carbon Nanotubes and Fullerenes (C_{60}) in Rainbow Trout (*Oncorhynchus mykiss* W.): Effects on Osmoregulation

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The rapid increase in production of carbon nanoparticles (CNPs) and the determination of their potential to impact on aquatic organisms is a prominent environmental concern, including exposure via the dietary route. This study investigated the chronic toxicity of single-walled carbon nanotubes (SWCNTs) and C_{60} fullerenes in juvenile rainbow trout exposed through the diet. The experiment consisted of two treatments (500 mg C_{60} kg^{-1} feed, 500 mg SWCNT kg^{-1} feed). Each CNP treatment and control (same feed but without CNPs) had three replicate tanks (100 L) in a semi-recirculating flow-through system. Fish were fed 2.8 % body weight per day for a 6-week exposure, followed by a 2-week recovery period (no CNPs in diet). Fish were sampled at time 0 and on weeks 2, 4, 6 (end of exposure) and 8 (end of recovery period). Fish length and weight were measured, blood was taken for haematology and organs were dissected for tissue ion concentration. Despite the relatively high exposure level of CNPs, there were no differences in mean fish length or weight between treatments and control during the exposure or recovery periods, indicating that CNPs did not affect fish growth. There were no consistent changes in haematology (blood cell counts, haemoglobin and plasma electrolytes). Plasma Na^+(control range 138-159 mmol l^{-1}, n = 30, all treatments range 129-167 mmol l^{-1} n = 46), plasma K^+(control range 3.37-5.84 mmol l^{-1}, n = 30, all treatments range 3.22-6.96 mmol l^{-1} n = 46) plasma osmolality (control range 274-311 mOsmol l^{-1}, n = 30, all treatments range 239-425 mOsmol l^{-1}, n = 46) and levels of tissue ions showed no consistent differences between the treatments and the control suggesting that CNPs in the diet did not impact fish osmoregulation. The question of whether uptake of CNPs can occur through the diet (i.e., is exposure occurring) and whether CNPs are accumulated within the tissues has yet to be addressed.

Toxicology of Dietary Titanium Dioxide Nanoparticles to Rainbow Trout, (*Oncorhynchus mykiss*)

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Our laboratory recently reported gut pathology following incidental ingestion of TiO_2 NPs during aqueous exposures in trout, but there are almost no data on dietary exposure to TiO_2 NPs in fish. The aim of this experiment was to observe the sub-lethal effects of dietary exposure to TiO_2 NPs in juvenile rainbow trout (*Oncorhynchus mykiss*). Stock solutions of dispersed TiO_2 NPs were prepared by sonication without the use of solvents and applied to a commercial trout diet. Fish were exposed in triplicate to either, control (no added TiO_2), 10, or 100 mg kg^{-1} TiO_2 NPs diets for 8 weeks followed by a 2 week recovery period where all fish were fed the control diet. TiO_2 NPs had no impact on growth or nutritional performance, and no major disturbances were observed in red or white blood cell counts, haematocrits, whole blood haemoglobin, or plasma Na^+. Ti accumulation occurred in the gill, gut, liver, brain and spleen during dietary TiO_2 exposure. Notably, some of these organs, especially the brain, did not clear Ti after exposure. The brain also showed disturbances to Cu and Zn levels (statistically significant at weeks 4 and 6; ANOVA or Kruskal-Wallis, $P < 0.05$) and a 50 % inhibition of Na^+K^+-ATPase activity during TiO_2 NP exposure. Na^+K^+-ATPase activity was unaffected in the gills and intestine. Total glutathione in the gills, intestine, liver and brain were not affected by dietary TiO_2 NPs, but thiobarbituric acid reactive substances (TBARS) showed up to 50 % decreases in the gill and intestine. We conclude that TiO_2 NPs behave like other toxic dietary metals where growth rate and haematology can be protected during sub-lethal exposures, but in the case of TiO_2 NPs this may be at the expense of critical organs such as the brain and the spleen. Pathologies in the spleen, brain, and liver are also reported.

Exposure and dose of engineered nanoparticles at working places

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Airborne engineered nanoparticles are the main health risk contributor at many workplaces especially in the nanotechnology industry. Deposition efficiency DE of nanoparticles in lungs is influenced by their size, e.g. DE =80% for 10 nm diameter particles and DE =15% for 100 nm diameter particles. Widely accepted metrics for health risk evaluation are based upon a fraction of airborne particle mass such as PM_{10}, $PM_{2.5}$. These metrics can cause uncertainties in health risk evaluations as they take no account of the effect of deposition efficiency, due to variation of particle sizes, on the exposure – dose relationship.

Exposure and dose have been evaluated using conventional OH sampling kit and a size resolving Wide Range Aerosol Sampling system (WRAS) at various working places. WRAS enables particle size distributions to be obtained across the entire airborne particle size range from 1 nm to 30 micro meter in diameter (www.naneum.com). These distributions were used to determine the exposure and the accumulated dose at working places. In parallel, samples were collected using conventional samplers. The uncertainties associated with the effect of size of nanoparticles on the exposure – dose relationship for Pb, Ag, carbon nano-tubes and silica were quantified.

The total mass concentration of nanoparticles and the nanoparticle mass fraction (i.e. sizes less than 100nm) varies considerably, e.g. for lead aerosols determined at working places mass concentration ranged from 0.6 □g/m^3 to 50 □g/m^3 and the mean size from 17 nm to 300nm. The nanoparticle mass fraction of aerosols was found to vary from 10% to 80%. The proportion of total mass of particles deposited in the respiratory tract of workers varied from 0.2 to 0.7. The evaluation of health risk based upon PM_{10}, $PM_{2.5}$ and other defined respirable fractions of the particle mass does not take account of levels of particle deposition in the respiratory tract and will therefore overestimate or underestimate the health risk considerably by up to a factor of 3.5.

Colloidal behaviour of nanoparticles in model natural fresh water, effect of natural organic matter

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The ecological risk assessment of chemicals and more recently of nanoparticles is based on the determination of adverse effects on organisms and on the environmental concentrations to which biota are exposed. We investigated the effect of different types of natural organic matter (NOM) on the behavior of nanoparticles in ultra pure water and algae medium. Significant differences in the stabilization efficiency of NOM from different sources and with nanoparticles of different composition, size and shape were observed at environmentally relevant conditions. This indicates that the transport and exposure of nanoparticles is greatly affected by interaction with natural organic matter. The interactions were more complex in model fresh water (algae medium) than in ultra pure water, as a result of the additional salts suggesting that detailed research is needed to understand the interplay of factors, and to identify the limitations of NOM stabilization.

Radio (^{14}C)- and Fluorescent- Doubly Labelled Silica Nanoparticles for Biological and Environmental Toxicity Assessment.

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A new and efficient synthetic route to fluorescent and ^{14}C double labelled silica based nanoparticles is described. The synthesis has been carried out using the “oil-in-water” micro-emulsion technique. Fluorescent and radioactive labelling has been achieved entrapping labelled poly(ethylene-glycol) molecules in the nanoparticles. The produced particles have been analyzed by means of scanning electron microscopy, photon correlation spectroscopy, confocal microscopy, scintillation counting and oxidation/combustion experiments. Fluorescence quenching experiments confirm that the label is entrapped in the particles. The results presented suggest that the silica matrix does not block the □-radiations emitted from the labelled PEG molecules entrapped in the NPs. Incubation experiments of an aqueous suspension of doubly labelled nanoparticles over twenty days have confirmed that the radioactive labelling remains in the core of the particles. The new doubly labelled NP can thus be used as a valuable tool for carrying fate and effect studies.

Discovery of protein markers for titanium dioxide nanoparticle –induced toxicity in mouse

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Titanium dioxide (TiO_2) nanoparticles have been widely used for commercial purposes, and the increase in consumptions of TiO_2 nanoparticles have been focused on their potential toxicity of human health and environmental impact. Recent investigations suggested that the inhalation of TiO_2 nanoparticles led to pulmonary toxicity and lung cancer. TiO_2 induced cytotoxicity was relevant to the size of particles. Although it is known that TiO_2 nanoparticles can induce serious pulmonary toxicities, the mechanism of the toxicity is not still unclear. In this study, we used a proteomic approach to compare the protein expression profiles in mouse following nano sized TiO_2 treatments, and further analyzed by using LC-MS/MS to identify the differentially expressed proteins. We also observed intracellular accumulation of nanoparticles and morphological damage by transmission electron microscopy. Analysis of 2-dimensional gel electrophoresis revealed that more than 1840 proteins spots were present in mouse lung. Among then, 13 proteins which showed up- or down regulated expression patterns in response to TiO_2 nanoparticle exposure were identified by using LC-MS/MS. The proteins may be candidates of the protein markers for TiO_2 nanoparticle –induced toxicity in mouse

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References:
*[1] H. W. Chen, S. F. Su, C. T. Chien, W. H. Lin, S. L. Yu, C. C. Chou, J. W. Chen and P. C. Yang, The FASEB Journal, **20**(2006) 2393-2395.*
*[2] S. J. Kang, B. M. Kim, Y. J. Lee and H. W. Chung, Environmental and Molecular Mutagenesis, **49**(2008) 399-405.*
*[3] J. J. Wang, J. S. Sanderson and H. Wang, Mutation Research, **628**(2007) 99-106.*

Iron oxide nanoparticles interact with fluorometric and colourometric dyes

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The reactivity of nanoparticles is often an unknown entity, consequently the possibility of direct interaction between nanoparticles and experimental assay components cannot be ignored, particularly when these tests are reporting on the nanoparticles' ability to induce cellular damage. Such interactions have the potential to result in false or misleading information. Some such instances have been documented in the literature, for example it has been shown that single walled carbon nanotubes interact with both fluorometric and colorimetric dyes, to give unexpected results when these probes are used for cell viability assays (Davoren et al. 2007; Herzog et al. 2007; Casey et al. 2008).

In the present study the fluorometric dyes dichloroflorescein (DCF) and 3'-(*p*-Aminophenyl) fluorescein (APF) were used to quantify the oxidative stress response induced by dextran coated ultrafine super paramagnetic iron oxide (Fe_2O_3 and Fe_3O_4) nanoparticles. In a cell free system increasing concentrations of dextran coated Fe_3O_4 nanoparticles (100ng/ml 1µg/ml, 10µg/ml, and 100µg/ml) induced a dose dependent decrease in DCF (2µM and 4µM) signal as compared to control levels. In contrast, the same concentrations of dextran coated Fe_2O_3 nanoparticles induced a dose dependent increase in DCF signalling. When the fluorogenic probe APF was used as an alternative to DCF, both Fe_2O_3 and Fe_3O_4 induced a dose dependent decrease in fluorescence. The present study also suggests that dextran coated iron oxide nanoparticles may directly interact with other fluorescent and colorimetric probes. For example, interactions between MTS (a colorimetric dye used for cell proliferation assays) and dextran coated Fe_2O_3 have also been observed in a cell free system, typified by an increase the colorimetric signal 2.5 fold as compared to control.

This study emphasises the importance of considering and controlling for possible interactions between nanoparticles and fluorometric or colorimetric dyes in experiments. Interestingly, the present study also draws attention to the importance of the oxidative state of iron oxide nanopartices in relation to their interactions with the fluorometric dyes as distinct differences were observed. Thus, where colorimetric or fluorometric dyes are to be relied on for experimental test systems, potential interactions with nanoparticles need to be considered if the investigators wish to reliably use these assays in a quantitative manner.

*Casey, A., E. Herzog, et al. (2008). "Single walled carbon nanotubes induce indirect cytotoxicity by medium depletion in A549 lung cells." Toxicol Lett **179**(2): 78-84.*
*Davoren, M., E. Herzog, et al. (2007). "In vitro toxicity evaluation of single walled carbon nanotubes on human A549 lung cells." Toxicol In Vitro **21**(3): 438-48.*
*Herzog, E., A. Casey, et al. (2007). "A new approach to the toxicity testing of carbon-based nanomaterials--the clonogenic assay." Toxicol Lett **174**(1-3): 49-60.*

High resolution imaging and *in vitro* investigation of fluorescent dye-modified TiO_2 nanoparticles toxicity in human primary keratinocytes

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Nanotechnologies are of great interest for researchers and industrialists, with numerous applications in domains such as medicine, catalysis and material sciences. However, their potential effects on human health have also attracted the attention of public and governments' worldwide. Established methods of chemical safety assessments have to be modified to address the special characteristics of nanoparticles and more especially to assess the biological effects of these highly reactive materials.

The main focus of this work is to provide a method where we have : (i) designed and synthesised fluorescent dye-modified titanium dioxide nanoparticles (TiO_2-NPs) using Fluorescein and Rhodamine, (ii) addressed the *in vitro* biological impact of both TiO_2-NPs and fluorescent dye-modified TiO_2-NPs on primary human keratinocyte cell populations, (iii) determined the intracellular accumulation and distribution at the single cell level using high resolution microscopy such as confocal microscopy (CM), electron microscopy (EM) and ion beam analysis (IBA).

We report here the first results of our investigations and show that fluorescent dye-modified TiO_2-NPs: **(i)** can be easily synthesised and used in molecular imaging as in immunocytochemistry; **(ii)** reproduce the cytotoxic effects as observed for the native TiO_2-NPs with a clear decrease rate in cell population doublings. Our results clearly stress a lower but significant toxicity of these fluorescent dye-modified TiO_2-NPs as compared to the bare ones. Furthermore, this toxicity is not related to the nature of the fluorescent dye used; **(iii)** are exclusively located in the cytoplasm and specifically accumulated in the peri nuclear region (CM, EM, IBA). Both fluorescent dye-modified TiO_2-NPs and TiO_2-NPs were found in different states: isolated NPs or entrapped in vacuoles or cytoplasmic compartments (EM). The use of ion beam analysis allows the quantification of the uptake of all TiO_2-NPs at the cellular level and the related cellular ion homeostasis.

These results show that such fluorescent dye-modified TiO_2-NPs combined with the last progress in genetics, biochemistry and molecular biology will facilitate the identification of impaired cellular function after exposure to NPs. These nano-objects, used in combination with high resolution fluorescence microscopy and video-microscopy on living mammalian cells or multicellular specimens (such as *in vitro* skin), will open new vistas in nanotoxicology analysis and will offer potential bio-imaging routine for the detection and tracking of NPs present in industrial products such as dermo-cosmetics.

Comprehensive evaluation of the behaviour of nanomaterials in solution: Suspension versus sedimentation

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Concern has been raised that exposure of humans to nanoparticles (NPs) might involve a significant health risk. A large number of nanotoxicological studies addressing this issue has been performed in vitro. In such studies it was often assumed without any proof that the nanostructured matter added to the cell culture media is suspended in the form of individual primary NPs with sizes according to the specifications of the manufacturer of the employed powder. Recently, however, Murdock et al. (Toxicol. Sci. 101 (2008) 239) have shown by dynamic light scattering (DLS) that most of the dissolved matter tends to form rather large agglomerates, even after having cracked aggregates by sonication. Hence one must expect that a sizable if not large fraction of the nanostructured matter will be subject to sedimentation. In such a case the idea of interpreting inflammatory or cytotoxic effects on the basis of the interaction of individual NPs with cells would be obsolete.

The aim of this work was to establish a solid basis for future in-vitro studies with adherent lung epithelial cells. To characterise the status of liquids doped with a nanostructured powder in a comprehensive manner, a large variety of analytical techniques had to be applied. In addition to (1) DLS we were using (2) wavelength-selective light absorption for determining the efficiency of sonication and the time dependent changes due to sedimentation, (3) optical microscopy to track the pile-up of agglomerates at the bottom of cell culture wells, (4) proton induced X-ray emission (PIXE) spectrometry to determine the NP content of µL quantities of medium extracted from the well at different heights and (5) scanning electron microscopy (SEM) to examine the morphology and agglomeration state of NPs in different sections of the liquid.

The results presented here relate to nanostructured powders of TiO_2 and Fe_2O_3 with specified primary particle sizes between 20 and 100 nm. Agglomeration was found to be a general phenomenon, being most pronounced in cell culture medium (HAM'S F12) without serum. Depending on the height of the liquid in the well, a sizable if not large fraction of the agglomerates was found to arrive at the bottom within 3 to 5h after introducing a freshly prepared solution. Whereas the agglomerates with sizes < 2µm exhibited long-term Brownian motion, even after days, the large agglomerates grown from the smaller ones were immobilised, attaining sizes well above 10 µm. At nominal mass concentrations exceeding about 20-30 µg/mL, the layer of agglomerates that had settled at the well bottom after about a day was visible by the naked eye. The mass concentrations detected in the supernatant, on the other hand, amount to only a few percent, or even less, of the as-prepared solution.

One of the important consequences of this work is that 'real' in-vitro studies with suspended NPs or small NP aggregates (< 200 nm) require prior removal of large agglomerates, e.g., by sedimentation.

Studies on the antibacterial properties of photo-irradiated Titania

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Photocatalytic property of semiconductor metal oxide titania (TiO2) is being extensively used for disinfection in applications like water purification, and floor cleaning devices. The principal objective of the present work was to study the effect of (a) concentration of TiO2, (b) exposure time to UV-C irradiation; (c) post irradiation time period, on antibacterial action by UV-C activated titania against two environmentally relevant microorganisms namely E.coli and P. aeruginosa. For exposure periods 15 mins, 30 mins, and 45 mins,. titania concentration was varied from 0.1 to 0.4 g/l to determine the EC50 concentration (the concentration at which the viable cell population comes down to 50% of that of the control). The photocatalyst concentration was increased further up to 0.8 g/l to study the saturation effect. The reversibility of the photocatalytic effect of TiO2 was studied through photocatalytic decay studies interacting irradiated titania kept in dark for different periods with the microorganisms and post irradiation experiments. Photocatalytic experiments with alternate light sources were also carried out to compare with UV-C results. The study outcomes will help to formulate an efficient photo killing strategy with TiO2 for domestic sterilization devices.

Keywords: Photocatalytic disinfection; TiO2; Post irradiation effect; Photocatalytic decay; Saturation effect; EC50 concentration.

Cell cycle-dependent localisation patterns of quantum dots.

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The cell cycle is the series of events that take place in a cell leading to its replication. The cell cycle is necessary for genetic maintenance and repair, and disruption of the cell cycle can lead to a number of pathologies such as tumour formation. Histone modification plays a crucial role in cell cycle replication. Previous work carried out in our centre showed that unmodified negatively charged CdTe quantum dots (QDs) display core tropism to core histones and histone rich cell organelles. However, the cell cycle response to unmodified luminescent semiconductor nanoparticles or quantum dots (QDs) is unknown.

To determine if QDs have an effect on cell cycle, the AGS gastric carcinoma cell line was treated with 5.2nm (red emitting) QDs over 24hrs. Cells were fixed and the effect of QDs on the cell cycle was identified by high content analysis (HCS).

To determine if QDs localised to different areas of the throughout the cell cycle, AGS gastric carcinoma cells were either serum starved to induce G1 phase block, or treated with nocodazole to induce a G2/M phase block, or left untreated for 16hrs before ethanol fixation. Cells were then permeabilised and incubated with 5.2nm QDs for 1hr. To identify cell cycle phase, nuclei were stained with Hoechst, a DNA intercalating agent, and cells imaged using confocal microscopy. To identify if the QD localisation was cell specific A549 airway epithelial cells were used as a control.

At a concentration of $1X10^{-6}$ M CdTe QDs significantly decrease cell viability in live AGS cells. At lower doses no increase in the G1:G2/M ratio of cells was seen with red QDs. Confocal microscopy showed clear cytoplasmic localisation of 5.2nm QDs in AGS cells. No QDs were found in nuclei at any stage of cell cycle. Distinct localisation patterns were identifiable between resting cells and those undergoing mitosis. Interestingly this was not specific only to the AGS gastric cell line, and similar localisation was evident in the A549 airway epithelial cell line. These results identify CdTe QDs as potential cell cycle markers in cell imaging assays.

Insignificant acute toxicity of TiO_2 nanoparticles to willow trees

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The environmental fate and effects of manufactured nanoparticles (MNP) are intensively studied due to their expected increase in production. Phytotoxicity of some types of nanoparticles has been observed for annual species. Yet, no results of toxicity tests with trees have been reported.

Trees account for the majority of biomass and are dominant constituents of several ecosystems. They consist of a large porous wood compartment, the secondary xylem, which is utilized to draw water and nutrients upwards from the roots. The size of the xylem vessels is in the micrometer range. Nanoparticles could block the xylem and so disturb its function. Thus, trees could be especially vulnerable to nanomaterials. This study tests the toxicity of TiO_2 nanoparticles on trees with the short-term willow tree transpiration test. TiO_2 particles with 25- and 100-nm diameter were suspended in distilled water at concentrations of 0, 1, 10, and 100 mg/L. Effects on transpiration, growth, and water use efficiency of exposed willow cuttings were monitored. The concentration of nanoparticles was measured by spectrophotometry.

None of the measured effect parameters showed any significant change during the test, indicating that willow trees were not sensitive to short-term exposure to TiO_2 nanoparticles in the conditions, concentrations, and time periods used in this study. Particles were rapidly lost from solution, probably due to sedimentation as a result of aggregation and also due to adsorption to roots. The preliminary results of this study should be confirmed with experiments using other types of MNP, other plant species, longer duration combined with lower exposure concentrations and experiments in soil, before a final conclusion in this issue can be made.

Mechanisms of protein binding to silver nanoparticles

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Silver nanoparticles (AgNP) are one of the most common nanomaterials in use today primarily because of their antimicrobial properties. Concerns regarding their eventual release into the environment are rooted in the fact that we still lack a complete understanding of their ecotoxicological effects in natural systems. Using a combination of matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry and chemical derivatization techniques, we studied the fundamental AgNP binding mechanisms of bacterial proteins purified from the bacterium *Escherichia coli*, other well-characterized model proteins, and synthetic peptides with a high silver-binding affinity. The presence of intense Ag^+ adduct signals in the MALDI spectra indicate very strong binding in certain peptic regions. When some acidic residues such as serine are shielded with a derivatizing agent, binding was not as specific and/or did not occur, suggesting these regions have a high affinity to AgNP. Using the same methodology, we found that nitrogen-rich amino acids such as histidine, lysine, and arginine bind preferentially if the AgNPs were coated with a carbonate shell and do not exhibit the same intense Ag^+ adduct signals indicative of strong association with AgNP. These results suggest a probable mechanism for protein adhesion to AgNP and may aid in our understanding of their ecotoxicological behavior on bacteria in the environment.

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