newsletter 3, september 2008

nano SAFE

Safe production and use of nanomaterials

News update of the Integrated Project NANOSAFE2

- ▸▸ **Detection, monitoring and characterization**
- ▸▸ **Health and hazard assessment**
- ▸▸ **Development of secure industrial production systems and safe applications**
- ▸▸ **Environmental and societal aspects**

Foto by courtesy of Nanogate/BGIA

European Integrated Project supported
through the Sixth Framework Programme
for Research and Technological Developement

Scope

The European integrated project NANOSAFE2 supported by the 6th framework programme of the European Commission is focussed on the safe production and use of nanomaterials. 25 Partners from industry, research centers and universities are developing new detection, monitoring and characterisation techniques as well as secure industrial production systems and safe applications of nanoparticles. The assessment of health effects, hazards, environmental as well as societal aspects is a further goal of the project.

NANOSAFE2 fits into the strategy of the action plan for nanosciences and nanotechnologies proposed by the European Commission. This strategy aims to responsibly integrate risk assessment related to human health, the environment, consumer and workers at all stages of the life cycle of nanotechnology, starting at the point of conception and including R&D, manufacturing, distribution, use and disposal or recycling.

The NANOSAFE2 project is embedded in global activities concerning safe nanomanufacturing and has close relations to other related projects on national, European and international level including the 6th framework programme integrated project SAPHIR (Safe Nanomanufacturing, www.saphir-project.eu), the French projects NACOMAT (Safe Nanomanufacturing of Advanced Ceramic Matrix Composites) and AMETIS (Safe Nanometallurgy) as well as the German project NanoCare (Responsible Use of Nanomaterials, www.nanopartikel.info)

Detection, monitoring and characterization

Nanoparticle monitoring at industrial sites

During a period of one year QNL has conducted air monitoring trials of nanopowder production as well as cleaning and maintenance activities in their production facility. Powder production measurements were conducted to determine the particle concentrations outside the facility simultaneously with measurements inside the facility in order to differentiate between background and engineered nanoparticle concentrations. However, it was found that background nanoparticle concentrations varied considerably and were strongly dependent on weather conditions (e.g. concentrations are considerably reduced after a period of heavy rain). Therefore, it was not possible to make a reliable distinction between particle concentrations of engineered nanoparticles and those present in the ambient air. It was also proved that the measured particle concentrations at QNL were well below workplace exposure limits set for conventional materials (e.g. fumed silica). Therefore, no further activities to minimize nanoparticle emissions from the production line were necessary.

CEA qualified an ink-jet workplace dedicated to the nanocoating of electrodes for battery applications. CEA used a new evaluation protocol based on preliminary helium measurements enabling the operators to locate optimized measurement points in the room (i.e. areas with low extraction efficiency). It was revealed that during the process particles > 100 nm and a few between 10 and 20 nm were generated. Particle level decreases drastically a few minutes after stopping the production process as it can be generally observed for processes with properly dimensioned ventilation. As a conclusion from the results of these measurements it was possible to reduce the safety delay time between the end of the pulverization process and personal re-entrance from hours to less than 10 minutes.

Figure 1
Ink-jet equipment pulverizing nanoparticles for battery electrodes (Source: CEA).

Development of online-measurement techniques

Measurement of high aspect ratio nanoparticles

Nanoparticle measurement devices like Scanning Mobility Particle Sizer (SMPS) and an Optical Particle Counter (OPC), which both rely on the assumption of spherical nanoparticles, have been investigated in order to determine how these techniques are applicable to high aspect ratio particles such as carbon nanotubes, nanowires, ribbon, etc. There have been measurements of multiwall carbon nanotubes (diameter 90 nm) with different lengths (300 nm and 900 nm) which were dispersed in the air using a rotating brush. With the SMPS, the short CNTs (L = 300 nm, d ~ 90 nm) were detected around 200 nm (equivalent sphere diameter) and the longer CNTs

(L = 900 nm) were detected around 250 nm. Artifacts of smaller equivalent sphere diameter were supposed to arise from broken CNTs. The OPC measurements were not very consistent and the optical equivalent sphere diameters of the MWCNTs varied between 200 - 500 nm or 700 nm - 2 µm.

Detection of nanoparticles in liquids

Five techniques were investigated for their ability to detect nanoparticles in liquids: Dynamic light scattering (DLS, Nanosight, Malvern Zetasizer), CPS Disc Centrifuge, electrophoresis (Malvern Zetasizer) and polyelectrolyte filters for TEM (Tunneling Electron Microscopy). All the investigated techniques proved to be suitable for the determination of nanoparticles in liquids. The most sensitive equipment was found to be the Nanosight instrument and the least sensitive was found to be the CPS Centrifuge. As to measuring number concentration and mass fraction of polydisperse nanoparticle samples, only the CPS Centrifuge technique yielded reliable results.

Cheap method for rapid detection of nanoparticles

First interesting results were obtained in the development of a cheap detector to identify big leaks in nanoparticle production lines using a modified smoke detector containing a radioactive source. A simplified prototype device employing Americium 241 alpha sources was build and tested with ZnO and TiO_2 nanoparticle aerosols. The results showed that the device is suitable for a very low cost detection of nanoparticles in a high concentration range.

Aerosol calibration device

VTT has further developed a calibration device which is able to produce aerosols with a nanoparticle concentration range between $3 \cdot 10^4$ and $9 \cdot 10^6$ particles per cm^3. They are now working on the miniaturization of the generator in order to get a portable tool.

Tracing and marking techniques

The CEA laser pyrolysis reactor has been equipped with a second chamber in order to add new functionalities to nanoparticles during their synthesis. The first chamber is dedicated to the production of nanoparticles by laser pyrolysis and, using two copper mirrors, the residual laser energy is reflected in the second chamber to allow an additional laser treatment of the nanoparticles. At this step, new reactants may be introduced to add e.g. luminescent nanotracers like silicon. The concept was first tested to achieve the attachment of silicon to the silicon carbide nanoparticles by introducing a flow of SiH_4 in the second chamber. Experiments show that in that process a thin coating around the silicon carbide nanoparticles has been formed, which will be further characterized in order to identify the exact composition.

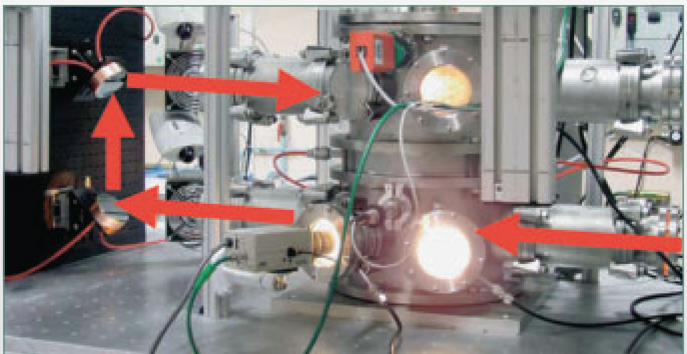

Figure 2
Double chamber reactor during a laser pyrolysis synthesis; the arrows indicate the optical path of the laser beam (Source: CEA).

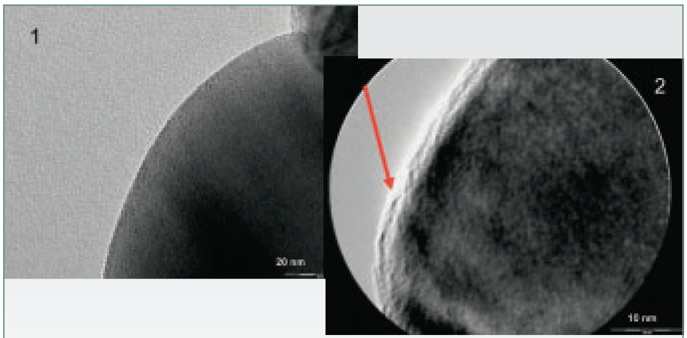

Figure 3
High resolution TEM micrograph of: 1) SiC nanoparticle synthesized after one pass through the laser beam. 2) SiC nanoparticle synthesized with two pass through the laser beam and the introduction of a silane flow; the arrow indicates a thin silicon coating of the nanoparticle (Source: CEA).

Tracing methods based on nanotracers and oligonucleotides

CEA designed a 3-color nanotracer able to contain about 100 different codes. These nanotracers ranging from 20 to 30 nm are made of organic molecular dyes inserted in synthetic organo clay presenting a hydrophobic part able to store large quantities of organic phosphor molecules. This leads both to intense fluorescence signals and a high recovery ratio during the synthesis which is lowering the fabrication costs. No quenching effect of the different colours was observed with this technology. The next step will consist in mixing nanocodes with nanoparticles and determine the low limit of detection of this labeling method for nanopowders in order to calculate the costs.

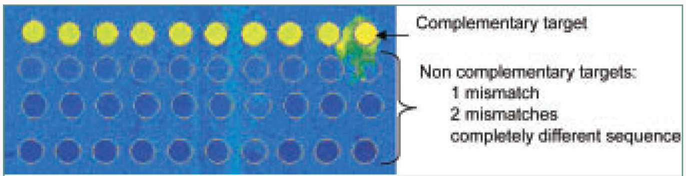

Figure 4
PCR Amplified DNA targets of DNA labelled CNTs detected on a DNA microarray. (Source: CEA).

Another approach using oligonucleotides as tracers for nanoparticles is progressed at CEA. During this study the main questions have been answered concerning the possibility of amplifying DNA of labeled nanoparticles, e.g. carbon nanotubes. Except for the dispersion rate of nanoparticles in an aqueous solution no real limitations became evident. A very large library of DNA coding sequences is thus available for labeling.

Sampling of nanoparticles in the gas phase

At VTT, an electron microscopy grid sampler based on nanoparticle diffusion and large particle thermophoretic repulsion (anti-thermophoresis) was redesigned

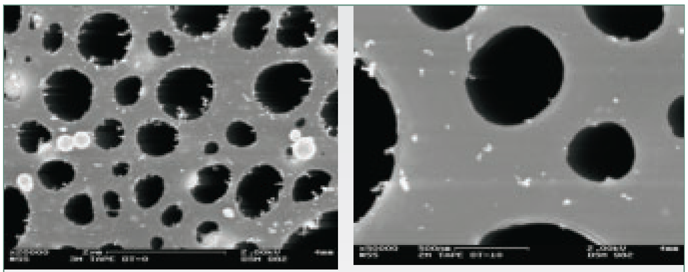

Figure 5
SEM images of TiO_2 particles collected with the heated EM grid sampler at optimal collection times. The grid was mounted on the heated resistor with a carbon tape, aerosol flow sweeping parallel to the surface of the grid. Left: Sampling time 3 min, temperature difference dT=0 °C, i.e. no thermophoretic repulsion. Right: Sampling time 2 min, temperature difference dT = 10 °C (Source: VTT).

Figure 6
Original Hartmann tube (left) and the modified closed system (right) to prevent nanoparticle exposure of operators (Source: SWISSI).

and made more compact. The grid is heated with a commercial, cylindrical shaped resistor heater. Based on the experimental results, a temperature difference of dT = 10 °C between the heated EM grid sampler and the passing aerosol flow is high enough to hinder large particle (dp > 200 nm) collection on the EM grid (see fig. 5). The performance of the heated EM grid sampler was compared to another individual particle sampler called aspiration sampler which is equipped with a preimpactor usually inserted in front of a TSI electrostatic classifier model 3080. The results from the comparison tests indicated no significant difference between these two samplers.

Characterization of nanopowders

Explosion severity

The explosion risks related to QNL aluminum nanopowder were assessed using a classical standard apparatus. Explosion severity was determined to be higher in the case of 200 nm aluminum nanopowder than in the case of 100 nm nanopowders. However, these experimental results should be handled with care as they may slightly underestimate explosion severity of an unconfined aluminum dust cloud. Consequently, the question on how to evaluate the explosion characteristics of non-passivated nanopowders without the effects of agglomerates remains unanswered.

Minimum ignition energy

SWISSI activities are concentrated on the development of devices dedicated to the estimation of safety parameters, especially the minimum ignition energy, the relaxation time of none conductive particles, powder bulk resistivity and friction sensitivity. The aim of these devices is to prevent an exposure of the operators to nanoparticles released during such testing. SWISSI developed a closed version of the classical open Hartmann tube to identify hazards due to electrostatics.

Limiting Oxygen Concentration (LOC)

The LOC defines the maximum oxygen concentration in a closed system to prevent an explosion by inerting in the presence of a ignition source. SWISSI performed such tests for non passivated aluminum nanopowders of QNL. A LOC of 3% has been estimated in nitrogen atmosphere, so for safety reasons the limit should be less than 1% in the production area.

Health and hazard assessment

Development of models for pulmonary absorption and body distribution

In the last 12 months of the project, the modeling team at INERIS re-analyzed the data of human exposure to Technetium-bound nanoparticles. The modeling was based on radioactivity data of blood, stomach, liver and urine in the time course of the experiments as well as gamma images of the experimental subjects at the end of the trials.

Development of experimental methods of in vivo whole-body distribution

Short-term inhalation studies with TiO_2, carbon black and carbon nanotubes (graphistrength C100 of ARKEMA) were carried out in male Wistar rats. TiO_2 was tested at the target concentrations 50, 10 and 2 mg/m^3, carbon black at 10, 2.5 and 0.5 mg/m^3, and graphistrength C100 at 2.5, 0.5 and 0.1 mg/m^3. Rats were exposed for 6 hours a day on 5 consecutive days. A concurrent control group was exposed to conditioned air. In those rats exposed to TiO_2, lung burden and possible translocation into other organs (liver, spleen, kidney, mediastinal lymph nodes, basal brain with olfactory bulb) were determined by ICP-AES immediately after exposure and after 2 weeks of recovery. The particles deposited in the lung were examined by electron microscopy.

In animals exposed to 50 mg/m^3 nano-TiO_2, a mean value (mean of 3 animals) of 1635 µg TiO_2 was found in the lung after the exposure, which decreased to 1340 µg after 2-weeks recovery. In animals exposed to carbon black, no treatment-related changes were found in the lavage fluid and in the blood. During necropsy, discoloration of the lungs was noticed in animals exposed to 10 mg/m^3. In animals exposed to graphistrength C100 changes of several parameters were found in the lung lavage fluid indicating inflammation processes at 0.5 and 2.5 mg/m^3.

Study via intravenous administration

The tissue distribution and toxicity of intravenously administered nanoparticles of TiO_2 (>80 wt% at <100 nm size) was investigated because of the fundamental importance

to obtain information on the kinetics of bioavailable nanoparticles. Male Wistar rats received a single intravenous injection of a suspension of TiO_2 in rat serum (5 mg/kg body weight). The tissue content of TiO_2 was determined 1, 14, and 28 days later. Biochemical parameters and antigens in serum were also assessed to determine potential pathological changes.
The health and behaviour of the animals were normal throughout the study. There were no detectable levels of TiO_2 in blood cells, plasma, brain, or lymph nodes. The highest TiO_2-levels occurred in the liver, followed in decreasing order by the levels in the spleen, lung, and kidney with the highest magnitude on day 1 in all organs. TiO_2 levels were retained in the liver for 28 days. There was a slight decrease in TiO_2 levels from day 1 to days 14 and 28 in the spleen, and a return to control levels by day 14 in the lung and kidney. There were no changes in the cytokines and enzymes measured in blood samples, indicating that there was no detectable inflammatory response or organ toxicity.

In vitro blood brain barrier translocations studies

The radiolabeled ARKEMA carbon nanotube material (0.1 microcuries per mL) as well as unlabeled nanoparticles (TiO_2, carbon black) at different concentration levels were introduced in the donor chamber (either luminal or abluminal compartment) of the in vitro rat BBB (Blood Brain Barrier) model test system. At time point 30 min after the addition of the radioactive medium aliquots were removed from the acceptor chamber (abluminal or luminal compartments) for radioactivity counting or nanoparticle determination by CPS centrifuge method. Experiments were done in triplicate for each nanoparticle.
No cell brain endothelial toxicity was observed at the concentration between 0,01 µg/mL and 10 µg/mL of nanoparticles (carbon nanotube, carbon black and TiO_2). The permeability for 14C carbon nanotubes was lower than the permeability of the paracellular marker sucrose indicating that no translocation of carbon nanotubes into the brain occurred.

Microfabricated system for in vitro nanoparticle testing

Microfluidics system

The approach of microfluidics fabrication using a polydimethylsiloxane (PDMS) substrate bonded to glass was continued. In previous periods, a major problem proved to be the bonding of the PDMS to the glass surface, which was frequently insufficient and not watertight. Tests were carried out on different bonding processes, including cleaning of the glass slide, activation of the PDMS with and oxygen plasma and heating after bringing the two elements into contact. A new process with an exceedingly improved sealing has been obtained. A gradient generator has been fabricated that can be used to create a dilution series from two starting solutions, and thus allowing to test a series of different nanoparticle concentrations in parallel.

Automated detection of nanoparticles

Following the results and the feedback from the first two years of the project, it was decided to focus the development work in the second year on the use of inductively coupled plasma mass spectroscopy (ICP-MS) for nanoparticle detection. One main task was to reduce the sample volumes to achieve compatibility with a microfluidics system. The sample injection method was changed to a TISIS (torch-integrated sample introduction nebuliser) linked to a peristaltic pump with flow meter. A low-volume cyclonic chamber was introduced instead of the previous cross-flow system and allowed for the reduction of sample volumes to 50 microlitres whilst sensitivity in terms of ppb detection limits remained very similar to the previous sample injection system. The data evaluation for sample sensitivity and injection conditions is still in progress.

In depth testing of the biological translocation system

To implement a test system for the translocation of nanoparticles in biological tissue CaLu-3 cells were grown on silicon nitride membranes. Although a good cell growth has been demonstrated the formation of a 'tight' monolayer with tight junctions has not been shown yet. First attempts to obtain tight junctions by immunostaining were unsuccessful. The interactions between particles and cells and the influence of the medium in which the particles are suspended were also evaluated. The interaction between particles and cells was tested with three particles: amine-modified quantum dots, carboxyl-modified quantum dots and non-functionalized quantum dots.

Integration of microfluidics and microfabricated wafers

A new generation of microfabricated silicon wafers with porous membranes was combined with the PDMS microfluidics elements to create the first 'integrated' system. A sandwich format was used with a central silicon chip, one PDMS element above and one below sealed to the silicon and a metal clamp holding the whole device together.

Development of secure industrial production systems and safe applications

CEA was able to further improve an automated liquid recovery system to collect nanoparticles formed by laser pyrolysis. Although it still needs some optimization efforts the principle has been profoundly demonstrated on industrial level. Mayor advantages of this technique are not only a safe recovery of nanoparticles but also the ability to disperse nanoparticles continuously leading to industrial relevant material varieties. In order to recover the TiO_2 nanoparticles produced by laser pyrolysis in water, studies were performed to optimize the dispersion and the stability of the suspensions. The influence of different parameters on the dispersion and the stability was studied. The powder characteristics produced after a dry or a liquid recovery were compared. The main difference concerns the carbon content rate which is much higher after a dry recovery while specific surface areas and density are almost the same.

Secure storage containers

ARKEMA has equipped several special drums of 60 litres with safety valves which consequently have been tested after several manipulations in order to approve the reliability of the system. CNT has to be transported from the production facilities to the packaging or application sites. In order to limit release, containers will be connected directly on the reception storage.
SWISSI has developed an experimental set-up to characterize the charge transferred and the energy dissipated in propagating brush discharges in laboratory experiments. Such discharges may be initiated during the pneumatic transport of nanoparticles through transfer pipes. The correlation found between the charges transferred in propagating brush discharges from polycarbonate foil and the geometrical arrangement depends on many different parameters which have to be investigated in the future.

Evaluation of nanoparticle release from end-products

VTT measured the possible release of nanoparticles during sol-gel coating processes. During the measurements, no increase in laboratory particle concentration was noticed which could be connected to the sol-gel process or grinding. Another VTT task was to test the dust accumulation of nanoparticles on sol-gel coated substrates. The surface energy, the topography and the gloss of the glass and steel substrates to be tested have been analysed. The results show that the sol-gel coatings decreased the surface free energy as compared to uncoated substrates. However, the coatings had minor effects on the surface roughness and on the gloss properties of the substrates. Preparations for the dust accumulation test have been made and a test chamber has been designed and constructed.

Nanogate investigated the occurrence of free flowing nanoparticles during industrial spray processes. Experiments have been performed with different materials and varying working conditions. All tests were done by BGIA implementing a simultaneous recording of the spray cloud with different CPC (Condensation Particle Counters) or other particle counter systems to achieve a high spatial resolution. The objectives of these experiments are to investigate the spreading distance of a potential nanoparticle cloud generated during the spraying process and if this cloud can be effectively removed by only a weak exhaust system.

Qualification of individual protection devices

The efficiency of commercial electrostatic filters to the graphite nanoparticles penetration was evaluated by CEA and BGIA. These results were compared with those obtained for fibrous HEPA filters. As expected, the electrostatic filter FPP3 showed a lower efficiency compared with a fiberglass HEPA filter tested at the same face velocity. For electrostatic filters, the MPPS (most penetrating particle size) in the nano range was observed to be around 30 nm. The protection efficiency of commercial gloves made of different material types and various thicknesses was evaluated. Gloves were exposed to graphite nanoparticles with a mean diameter around 40 nm. No penetration of nanoparticles through the gloves was observed.

Figure 7
Simultaneous measurements of the spray cloud at Nanogate in a simulated environment with different CPC (Condensation Particle Counters) run by BGIA (Source: BGIA).

Environmental and societal aspects

Standardization and regulations

The Nanosafe project partners contributed actively to the current relevant standardization activities of ISO TC 229 and CEN TC 352 Nanotechnologies. A couple of technical reports and technical specifications have been published or are in preparation, e.g.

- Use of Transmission Electron Microscopy (TEM) in the Characterization of Single Walled Carbon Nanotubes (SWCNTs) (Technical specification)
- Use of Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analysis (EDXA) in the Characterization of Single Walled Carbon Nanotubes (SWCNTs) (Technical specification)
- Use of UV-VIS-NIR absorption spectroscopy in the Characterization of Single-Walled Carbon Nanotubes (SWCNTs) (Technical specification)
- Use of NIR-Photoluminescence (NIR-PL) Spectroscopy in the Characterization of Single-Walled Carbon Nanotubes (SWCNTs) (Technical specification)
- Measurement Methods for the Characterization of Multi-Walled Carbon Nanotubes (MWCNTs) (Technical specification)
- Best practice procedures for production and use in work places (Technical report to be published in 2008)

Several contacts with ISO and CEN technical groups have been made to improve current standards or propose new methodologies.

Standardization and regulations

Life Cycle Analyses

Life Cycle Analyses of selected nanoparticle-based products have been continued. An in-depth review has been conducted based on available information regarding the fabrication, use and disposal of vehicle tyres, with special attention to the differing effects of using carbon black, carbon nanotubes, and silica nanoparticles as filler materials. One key question addressed was the effect of different filler particles on the tyre wear rate, as research at VTT has shown that tyre wear gives rise to atmospheric nanoparticles. As it is difficult to obtain data on the consequences of such nanoparticle emissions on the environment the final report on this work will have to present a variety of different scenarios with different assumptions about the environmental consequences.

Training

A wide range of presentation and training activities on the safety aspects of nanomaterials have been conducted on international level. A new internet portal "Nanosafe Education" is being constructed which will help to train staff for a safe management of nanomaterials, to improve communication with the public and to develop societal acceptability of nanotechnology.

Dissemination

A lot of dissemination activities have been performed during the project, i.e. distribution of leaflets and newsletters, presentations at conferences, as well as the continuous updating of the website "nanosafe.org". In order to make detailed information available to the public, technical reports addressing relevant topics have also been distributed. So far, two examples of dissemination reports have been issued

- Safety Parameter Characterisation Techniques for Nanoparticles, (INERIS)
- Efficiency of Fibrous Filters and Personal Equipment against Nanoaerosols (CEA)

Figure 8
Examples of technical reports issued by the Nanosafe project (see www.nanosafe.org).

NANOSAFE2 partners

- ARKEMA (www.arkema.com)
- BASF AG (www.basf.com)
- BGIA (HAUPTVERBAND DER GEWERBLICHEN BERUFSGENOSSENSCHAFTEN, www.hvbg.de)
- CAESAR (STIFTUNG CAESAR, www.caesar.de)
- CEA (COMMISSARIAT À L'ENERGIE ATOMIQUE, www.cea.fr)
- CSEM (CENTRE SUISSE D'ELECTRONIQUE ET MICROTECHNIQUE S.A., www.csem.ch)
- DEKATI OY (www.dekati.com)
- DG TEC S.A.S (www.dgtec.fr)
- ECRIN (ECHANGE ET COORDINATION RECHERCHE INDUSTRIE, www.ecrin.asso.fr)
- GSF (FORSCHUNGSZENTRUM FÜR UMWELT UND GESUNDHEIT GMBH, www.gsf.de)
- HSL (HEALTH AND SAFETY LABORATORY, www.hsl.gov.uk)
- INERIS (INSTITUT NATIONAL DE L'ENVIRONNEMENT INDUSTRIEL ET DES RISQUES, www.ineris.fr)
- INSERM (INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE, www.inserm.fr)
- JSI (JOZEF STEFAN INSTITUTE, www.ijs.si)
- KUL (KATHOLIEKE UNIVERSITEIT LEUVEN, www.kuleuven.ac.be)
- NANOGATE ADVANCED MATERIALS GMBH (www.nanogate.com)
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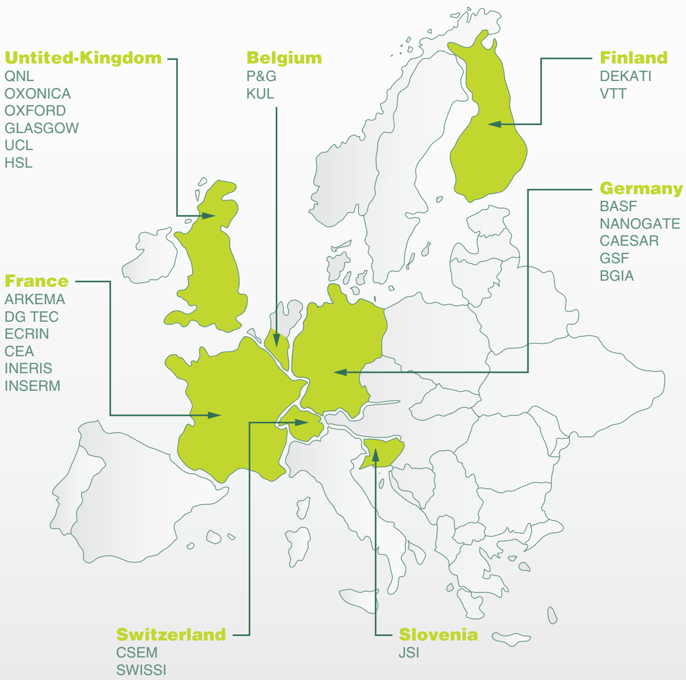

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- UCL (UNIVERSITY COLLEGE LONDON, www.ucl.ac.uk)
- UNIVERSITY OF GLASGOW (www.gla.ac.uk)
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Impress

Published by
Zukünftige Technologien Consulting (ZTC)
VDI Technologiezentrum GmbH
Peter-Müller-Straße 1
40468 Düsseldorf (Germany)
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Upcoming Nano-Meetings

nanosafe 2008
3 - 7 November 2008, Grenoble, France
www.nanosafe.org/

Nanotoxicology: Health & Environmental Impacts
27 February 2009, Hearfortshire, Great Britain
www.regonline.co.uk/nano09

6th International Conference on Biomedical Applications of Nanotechnology
4 - 6 March 2009, Berlin, Germany
http://nm09.nanoevents.de/index.php

Viennano '09 – 3rd Vienna International Conference on Nano Technology
18 - 20 March 2009, Vienna, Austria
www.oetg.at/viennano09/

Conference NanoImpactNet – for a healthy environment in a future with Nanotechnology
23 - 27 March 2009, Lausanne, Switzerland
www.nanoimpactnet.eu

International Conference on the Environmental Implications and Applications of Nanotechnology
9 - 11 June 2009, Amherst (MA), USA
www.umass.edu/tei/conferences/NanoConference/index.html

4th International Conference on Nanotechnology – Occupational and Environmental Health
26 - 29 August 2009, Helsinki, Finland
www.ttl.fi/Internet/English/Information/International+meetings+and+symposia/Nanoeh2009/