newsletter 2, february 2008

nanoSAFE

Safe production and use of nanomaterials

News update of the Integrated Project NANOSAFE2

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 techniques

Monitoring of nanoparticles at industrial sites

One important aspect of the NANOSAFE2 project is the monitoring of the nanoparticle exposure at workplaces in industrial production sites or in laboratories. To improve the knowledge on nanoparticle backgrund levels, which can strongly interfere with nanoparticle exposure detection, long term measurements in an industrial workplace, laboratory rooms and in clean rooms have been performed by QNL and CEA. CEA has measured nanoparticle background levels with a SMPS-C (Sequential Mobility Particle Sizer – Classifier) equipped with an aerosol neutralizer. A big variation of nanoparticle background levels were detected in a conventional laboratory room as shown in figure 1. This illustrates the difficulty of the detection of engineered nanoparticles which normally can not be discriminated from background particles, and highlights the demand for specific detection tools.

To assess the workplace exposure of carbon nanotubes at a production site of ARKEMA CEA and INERIS performed measurements using online techniques such as SMPS and off-line techniques like Andersen impactor and quartz fibre filters, which were subsequently analyzed with ICP-MS and Electron microscopy. First results indicate that under certain circumstances fine CNT aerosols are generated while handling the CNT materials.

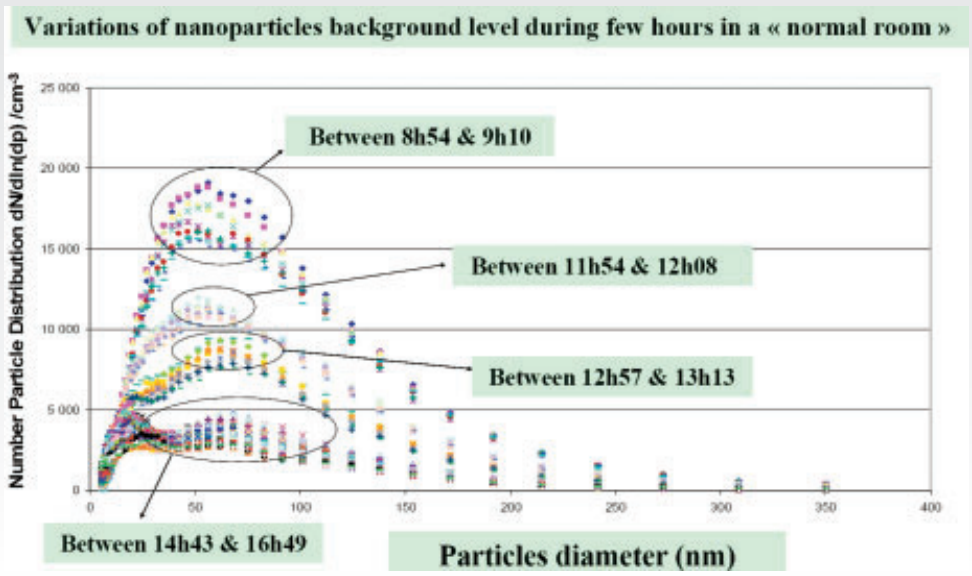


Figure 1
Variations of nanoparticle background level during few hours in a normal laboratory room without nanoparticle generation.

Development and evaluation of on-line monitoring techniques

Gas Phase Detection

QNL has evaluated a range of instruments for on-line particle size measurement. Trials were conducted using commercial available devices from TSI. Because of problems in the measurement process a more suitable detection method was developed which consists of a separate Differential Mobile Analyser and Condensation Particle Counter. In order to validate the instrument and avoid interrupting the production, a powder containment chamber was developed to simulate the processing conditions. With this setup it is possible to disperse nanopowders at controlled rates into the chamber that allowed the particle concentrations and particle size distribution to be measured (See figure 2). The results of the trials verified that the instruments could be used for on-line particle measurement.

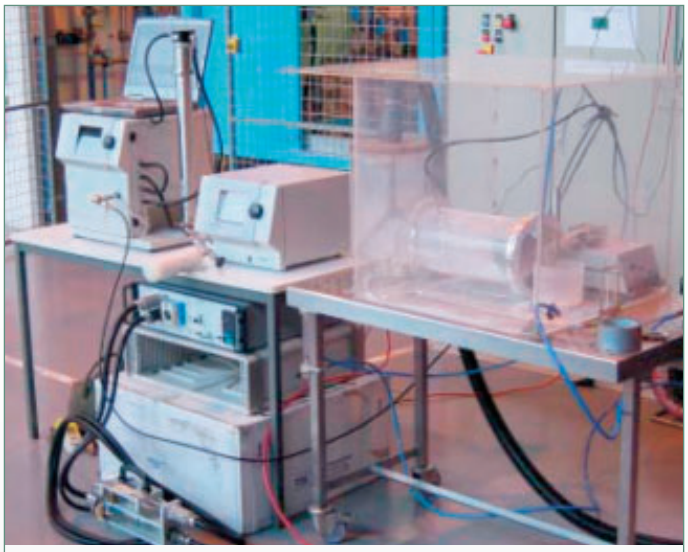


Figure 2
Powder containment chamber used to evaluate the DMA and CPC system supplied by TSI

Liquid phase detection

CAESAR is developing a peptide based biosensor for detecting nanoparticles in liquid phases. Several peptides were sequenced and screened for nanoparticle binding capabilities. A suitable DNA construct has been designed that will allow selection of peptides from a library of about 10^{12} different sequences resulting in a much better coverage of the sequence space of possible peptide binders. Currently experiments are planned to use this system to find binders for TiO_2 and gold nanoparticles. At Oxford, the light scattering-based techniques used in the Malvern Zetasizer continue to be the main means of detecting and determining the size of particles suspended in liquids. Also a new Nanosight particle detector based on light scattering from particles undergoing Brownian motion has been evaluated. Because of its high sensitivity it will probably be the technique of choice for the detection of very low levels of nanoparticles in liquids.

Tracing and marking techniques

Tracing and marking technologies are key components for nanoparticle monitoring programmes. In the project solid particles of fluorescein and YVO_4Eu particles as a mineral tracer with a good thermal stability were selected as marker substances. A portable decoding system based on a microspectrometer and a PDA to interpret the optical nanotag has been developed to detect the mineral nanotracers (See figure 3). Furthermore, a new approach using organic tracers has been set up for nanoparticle applications which cannot accept foreign elements (e.g. microelectronics). In this case the tracer can be removed from the nanoparticles just before their final use by a simple thermal or chemical treatment. This “Post it” method designed at CEA uses 1-chlorohexan as a chemical tracer for nanoparticles, which is detectable by GC-ECD. Currently the feasibility of 1-chlorohexan is demonstrated for marking carbon nanotubes.

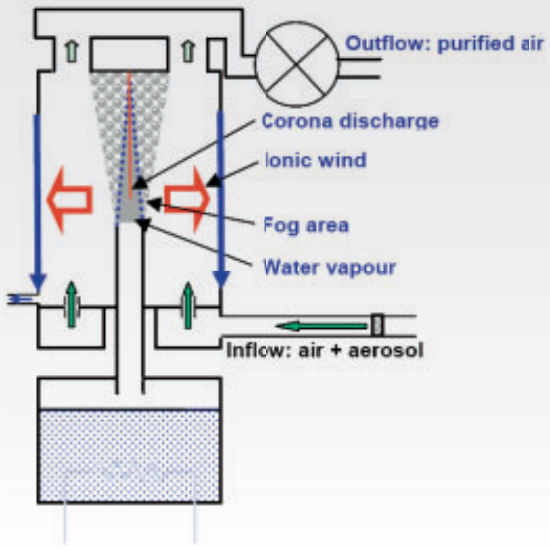
Off line monitoring and sampling techniques

Several nanoparticle sampling techniques related to the new particle detection techniques have been developed and evaluated using different physical principles such as electrostatics, thermophoresis, bubbling, vapour condensation, etc. CEA developed a new type of aerosol sampler from air to water based on electrostatic attraction. The advantage of this technique is the capture yield enhancement, because of the large amount of sampled air compared to the small collection liquid volume where the particle content of the air is concentrated. Therefore, this capture device is particularly adapted to continuous flow monitoring by fluidic microsystems. The collection device consists in a standard electrostatic precipitator, where the particle are charged via an electrical corona discharge and collected subsequently due to the electrostatic forces (See figure 4).

Figure 3
Decoding device for optical nanotags

Physico-chemical and safety parameter characterization

A wide spectrum of analytical techniques have been applied for the physical characterisation of the four particle types selected in the NANOSAFE2-Project (TiO_2, carbon black, carbon nanotubes and aluminum). This includes but is not limited to SEM, TEM, X-ray analysis, PCS (Photon Correlation Spectroscopy), BET, calorimetry, Picnometry and Zetametry. These investigations have been completed by additional characterisations of nanoparticles in biological media. The characterisation data will be correlated with the results of the toxicology testing and will be a basis to draw conclusions from physical properties of nanoparticles to their toxicological behaviour.



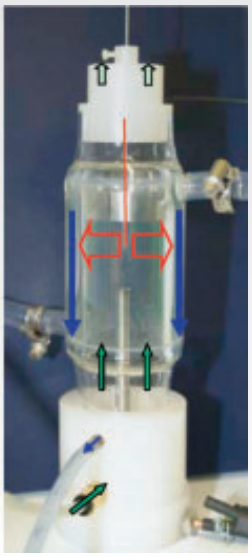


Figure 4
Schematic diagram of the collection device (left) and a photography of the fabricated device (right)

Health and hazard assessment

Cytotoxicity testing

An important task of the Nanosafe2-project is to develop screening techniques to detect possible health effects of nanomaterials. Meanwhile two cytoxicity tests on two human cell lines have been established and validated. Cytotoxicity data we obtained with reference materials (multi walled carbon nanotubes, titanium dioxide and carbon black) as well as approximately 30 non reference nanoparticles. For the reference materials no or only weak cytotoxic effects were observed.

Development of experimental methods in vivo whole-body distribution*

For the evaluation of the inhalation toxicity of nanomaterials technical setups for generation and characterization of test atmospheres were established. Nano-sized titanium dioxide (TiO_2) and carbon black have been aerosolized and their agglomeration state and potential to release nanoparticles have been investigated. Prior to aerosolisation, the particle size, specific surface area, zeta potential and morphology of each test substances were determined. Although the aersosol generation used relatively high energy, most of the particles in the atmospheres were found to be agglomerates of nanoparticles and no more than a few mass percent of particles below 100 nm were present in the aerosols.
In a second step, a short-term inhalation study with nano-TiO_2 and carbon black were carried out in rats. Rats were exposed various concentrations of nano-TiO_2 carbon black for 6 hours a day on 5 consecutive days; pigmentary TiO_2 served as a non-nano reference substance. A concurrent control group was exposed to conditioned air. For TiO_2 Lung burdens 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 two weeks of recovery. The particles deposited in the lung were examined by electron microscopy. Concerning particle deposition in the lung mostly large agglomerates of up to 2.5 micron in diameter were found which corresponds to the particle sizes of the inhaled aerosol. There were no signs of desagglomeration in the lung. In the liver, kidney, spleen and basal brain with olfactory bulb, no TiO_2 could be detected (detection limit 0.5 micrograms/tissue).
Additionally the tissue distribution of intravenously administered nanoparticles of TiO_2 was investigated to obtain information on the kinetics of nanoparticle in a situation of 100 % bio-availability. Male Wistar rats were treated with single intravenous injections of a suspension of TiO_2 in serum (5 mg/kg body weight), and the tissue content of TiO_2 was determined 1, 14, and 28 days later. There were no detectable levels of TiO_2 in blood cells, plasma, brain, or lymph nodes. The TiO_2 levels were highest in the liver, followed in decreasing order by the levels in the spleen, lung, and kidney, and highest 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.

Development of a microfabricated miniaturized system to test nanoparticles in vitro

The aim of this workpackage is to develop a microfabricated device as an in vitro screening method to accurately assess the potential of specific nanomaterials to cross through the lung epithelium into the blood. The final goal is to determine safety parameters for several types of nanomaterials that are commercially produced or occur during fabrication. If successfully implemented this tool could also be used to help anticipate the health impact of nanoparticles on the human body. In the first year of the project, work in this task concentrated on studying different biological models and choosing the most suitable for work with the microfabricated device. The Calu-3 cell line was found to give the 'tightest' cell layers and best results in translocation tests done using conventional porous membranes and fluorescent polystyrene nanoparticles. Additionally first steps have been made to develop the different components of the microfabricated device such as a microfabricated cell culture plate, TEER (transepithelial electrical resistance) electrodes to measure the tightness of the layers and the microfluidic components. To aid the development of the system, cadmium sulphide fluorescent quantum dots have been synthesized as a convenient water soluble type of nanoparticle which is simple to detect (using fluorescence microscopy), and can be adjusted for size, and weight. These dots will be used to evaluate fluidics elements, fluid flow, the formation of gradients etc. For the development of an automated nanoparticle detection system several techniques have been investigated for suitability such as dark field microscopy and inductively coupled plasma mass spectroscopy.

Development of safe industrial production systems and applications

Limitation of nanoparticles release at source level

CEA was able to develop an automated liquid recovery system in order to transfer nanoparticles formed by laser pyrolysis. The principle has been profoundly demonstrated on industrial level as a safe method to capture nanoparticles directly after their creation and should be also portable to other related processing routes such as the industrially widespread flame pyrolysis.
The liquid recovery/collection system has been installed at the beginning of the second year of the project. The equipment is connected to the laser pyrolysis reactor as shown in figure 5. The equipment has been designed to test several functionalities for the slurrying of nanoparticles produced by gas-phase processes such as laser pyrolysis. When the flow of nanoparticles is entering the equipment, it is possible to achieve the dispersion of nanoparticles by bubbling, mixing of liquid in the flow of nanoparticles and using also an "in-situ" cleaning to remove nanoparticles agglomerated on filters. The dispersion of nanoparticles is enhanced by "in-situ" ultrasonic treatments and using a recirculation pump for the stirring.
A somewhat more straightforward approach to identify free flowing nanoparticles was undertaken by a combined approach of Nanogate and HVBG-BGIA. Nanogate performed several typical production scenarios where possibly free nanoparticles can be produced or can be set free into the environment. These handling procedures include thermal treatment, filling and emptying furnace reaction chambers as well as mixing nanomaterials. However tests by HVBG-BIA showed that in not a single case of the extended measurements carried out, a higher concentration of nanoparticles was found than in uncontaminated office air. Therefore the risk of the release of free flowing nanoparticles due to handling procedures of nanomaterials seems to be quite low. However, there are processes such as the spray pyrolysis of

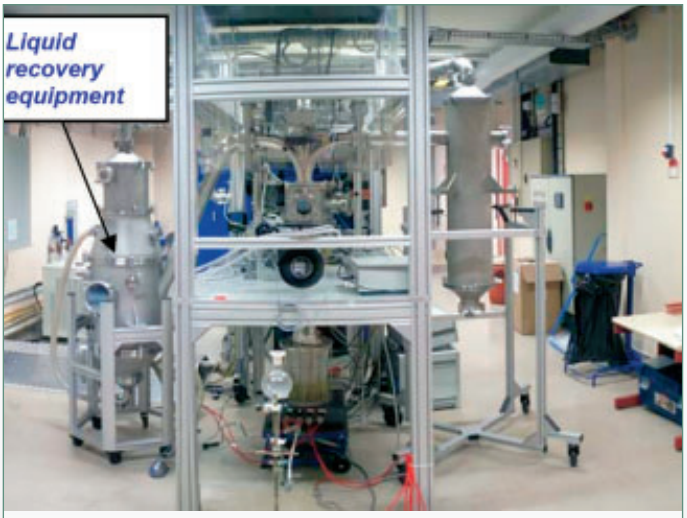


Figure 5
Liquid recovery/collection equipment connected to the laser pyrolysis reactor.

silane-based materials quite often used in paint shops for enhancing the adhesion of coatings onto sensitive substrate surfaces. It could be shown in an exemplary set up that silanes, when exposed to a thermal treatment may form free flowing SiOx nanoparticles. Consequently, Nanogate will extend their measurements on these production situations in order to minimise the potential risks. Another aim of this work package is to evaluate safe storage conditions of nanoparticles in transport and storage containers. Some investigations have been performed concerning the tightness of plastic containers as well as the self ignition temperature of nanoparticles and -tubes. These experiments will generate valuable data

to avoid hazards of nanoparticle leakage and explosions during storage.
Also the release of nanoparticles from end-user products and processes has been evaluated. For example VTT measured the possible release of nanoparticles during sol-gel coating processes. The measurements were done in December 2006 in VTT material laboratory, Tampere. During the measurements no release and transport of nanoparticles originating from the coating process to the laboratory air was observed.

Nanoparticles confinements and protections at working places

Extensive work was done to evaluate the workers protection against nanoparticle exposure and to investigate appropriate filter materials. As a first result it can be stated that some of the currently available filter materials seem to deliver a reasonable barrier against free flowing sample nanoparticles. Despite of the fact that more and extensive tests especially concerning high nanoparticle concentrations have to be carried out, the obtained results showed that classical protection means like HEPA filters are effective tools to protect for nanoparticle exposure. CEA designed a bench test to evaluate protection clothes and gloves. As another approach to avoid worker exposure with nanoparticles an automated production line for nanoparticle production will be developed within the project. First, the work concerned the centralized monitoring of all the equipments of the line (valves control, reactant flow rate control, pressure regulation, power monitoring) from a unique computer located in a control-command room. It concerned also the definition of safe procedures for the monitoring of the process. At the end of the second year, it is possible to control the injection of reactants and the opening and closing of valves from the computer. The program calculates also the amount of energy absorbed by the reactants. During the third year of the project, the work will be continued in order to be able to manage all the installation from the computer. For example, it is planned to implement the control of the opening and closing of the valves for the injection of gaseous reactant. It is also planned to start a basic implementation of an automatic security chain, in case of accidental laser shutdown for example or of accidental window failure.

Monitoring Chains

The setting up of industrial monitoring chains goes along with the completion of the production equipment of the NANOSAFE2 partners being developed in other work packages. To test and develop methods and equipment adapted to carbon nanotubes characterization at workplace Arkema has established a room dedicated to nanotubes handling. Samplings at workplace atmosphere are carried out in the handling room. An operator, equipped with an overpressured suit, makes different operation with crude CNT and precomposite in order to have an actual release of CNT (Emptying a drum with a shovel, sieving, transfer between containers equipped with high containment valves). Samples collected during the monitoring campaign will be controlled by scanning electronic microscopy and transmission electron microscopy, in order to detect carbon nanotubes and compare the efficiency of the different sampling techniques.

Figure 6
Operator in a secured handling room for nanomaterials

Environmental and societal aspects

Development of a societal risk assessment methodology at work places

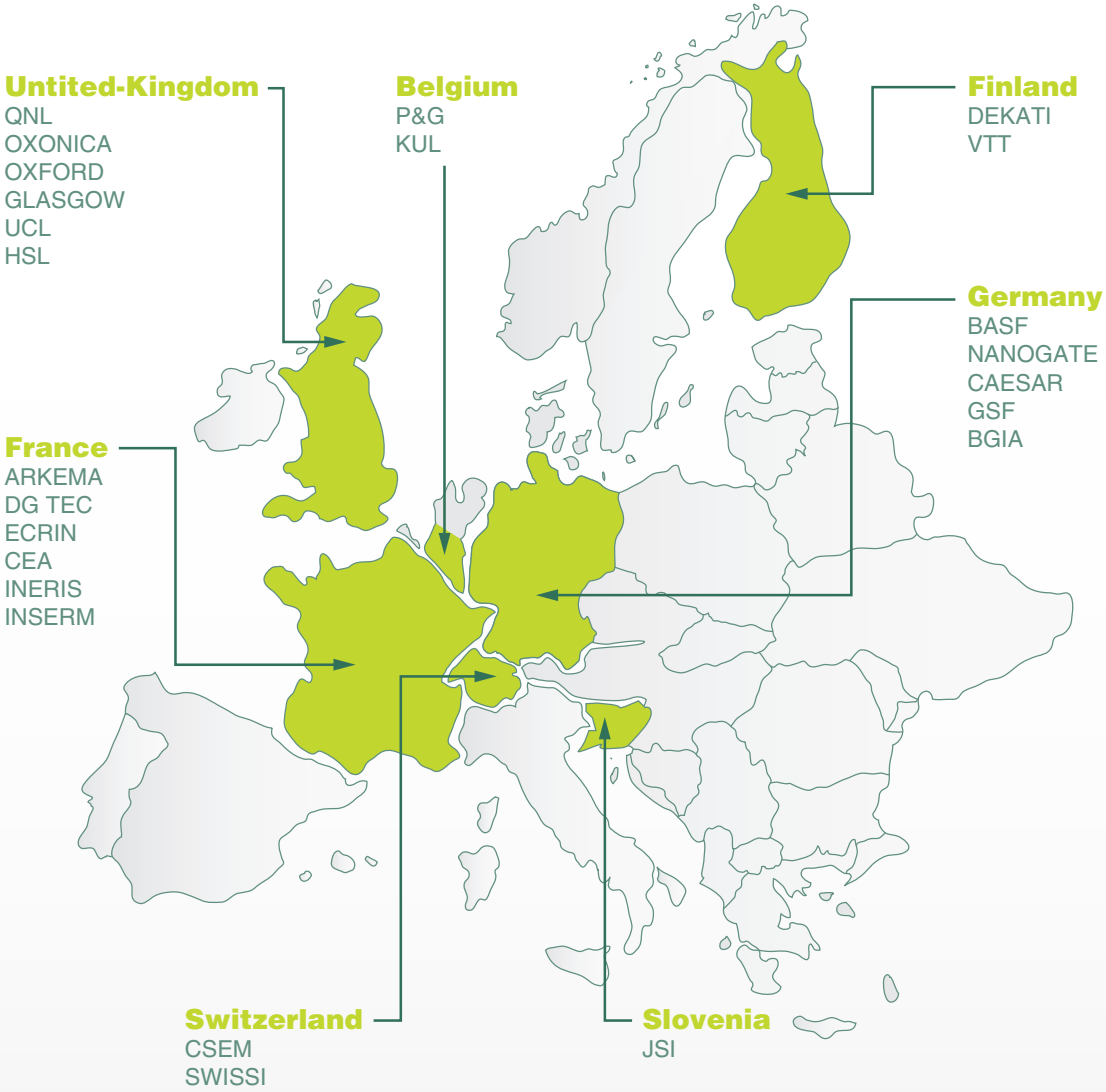
Dust at the workplace is responsible for serious and disabling diseases like pneumoconiosis, interstitial lung disease and fibrosis, lung cancer and asthma. The potential hazard of different nanomaterials is not predictable by the bulk physico-chemical properties and detailed data on exposure assessment are still lacking. An epidemiological literature survey by the GSF found that the size of the effects is often larger for ultrafine than for fine particles on a per mass basis. Lung diseases attributed to environmental air pollution are: deterioration of lung function and respiratory symptoms, exacerbations of chronic obstructive lung disease, asthma and allergies; and lung cancer. In addition, several mechanisms have been hypothesized to contribute to deaths from cardiovascular diseases. The inhalation of particles provokes oxidative stress and triggers systemic inflammation leading to altered blood rheology favoring coagulation; vascular dysfunction; and enhanced atherosclerosis – all increasing the risk of a subsequent myocardial infarction. The alteration of the autonomic nervous control of the heart increases the likelihood of ischemic heart deseases and cardiac arrhythmias.

Life Cycle Analysis

Progress has been made in understanding the principles and practicalities of life cycle analysis. A questionnaire has been designed to be piloted with ARKEMA, which is the key factor in assembling the life cycle inventory. Freely available existing databases will form the basis for the life cycle analysis that will be completed by environmental impact data generated later in the course of the NANOSAFE2 project. The software tool CMLCA is being tested for the initial assembly of our life cycle analysis test case.

Training Within the activities to implement a training strategy at academic levels a general course on nanotechnology was tested at a pilot scale consisting of 5 volunteers at Oxford and with. 12 more people having registered for a full master course. A two-hour course in an on-going training module designed for occupational safety officers is being proposed at HSL and BGIA. INERIS is elaborating and consolidating the contents of this course by integrating all nanosafety related materials gained during this project.

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)
- OXONICA LTD (www.oxonica.com)
- P&G (PROCTER & GAMBLE EUROPE, www.eu.pg.com)
- QINETIQ NANOMATERIALS LTD (www.qinetiq.com)
- SWISSI (INSTITUTE FOR THE PROMOTION OF SAFETY AND SECURITY, www.swissi.ch)
- UCL (UNIVERSITY COLLEGE LONDON, www.ucl.ac.uk)
- UNIVERSITY OF GLASGOW (www.gla.ac.uk)
- UNIVERSITY OF OXFORD (www.ox.ac.uk)
- VTT (TECHNICAL RESEARCH CENTRE OF FINLAND, www.vtt.fi)

VDI Technologiezentrum

Impress

Published by
Zukünftige Technologien Consulting (ZTC)
VDI Technologiezentrum GmbH
Graf-Recke-Straße 84
40239 Düsseldorf (Germany)
www.zt-consulting.de

Dr.Dr. Axel Zweck

Contact
Dr. Wolfgang Luther
Phone: +49 (0) 211 62 14-5 82
Fax: +49 (0) 211 62 14-1 39
Email: luther@vdi.de

Layout
Jascha Knoche
knoche@vdi.de

www.nanosafe.org

Project leader
Frédéric Schuster (CEA, F)
frederic.schuster@cea.fr

Subprojects leaders
Detection, monitoring and characterization techniques
François Tardif (CEA, F)
francois.tardif@cea.fr

Health and hazard assessment
Peter Hoet (KUL, B)
peter.hoet@med.kuleuven.be

Development of safe industrial production systems and applications
Martin Klenke (Nanogate, D)
martin.klenke@nanogate.com

Environmental and societal aspects
Jacques Bouillard (Ineris, F)
jacques.bouillard@ineris.fr

EC Project officer
Georges Katalagarianakis

Upcoming Nano-Meetings

1st Annual Conference on Nanotechnology Law, Regulation and Policy
28 - 29 February 2008, Washington D.C., USA
www.fdli.org/conf/431

nanoECO
2 - 7 March 2008, Monte Verita, Switzerland
www.empa.ch/nanoECO

Nanotechnology and Toxicology in Environment and Health
2 - 3 April 2008, Leipzig, Germany
www.ufz.de/index.php?de=15621

Risk, Uncertainty and Decision Analysis for Nanomaterials: Environmental Risks and Benefits and Emerging Consumer Products
27 - 30 April 2008, Portugal
www.risk-trace.com/portugal2008/

Nanotoxicology – 2nd International Conference
7 - 10 September 2008, St. Gallen, Switzerland
www.nanotox2008.ch

NanoRegulation 2008
16 - 18 September 2008, St. Gallen, Switzerland
www.nanoregulation.ch

Nanorisk 2008
21 - 24 October 2008, Paris, France
www.upperside.fr/nanorisk2008/nanorisk2008intro.htm

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