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November
13-15
MINATEC
Grenoble France 2012

BOOK OF ABSTRACTS

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SAFE'12

THIRD INTERNATIONAL
CONFERENCE
ON SAFE PRODUCTION AND
USE OF NANOMATERIALS

MINATEC, GRENOBLE, FRANCE

Book of Abstracts

**INTERNATIONAL CONFERENCE ON
SAFE PRODUCTION AND USE OF
NANOMATERIALS**

MINATEC, GRENOBLE, FRANCE

13-15 November 2012

NANOSAFE 2012

Conference Organization

Local Organizing Committee

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Ken TAKEDA (U. TOKYO, JP)
Olivier WITSCHGER (INRS, FR)

Welcome from the organizers

The rapidly developing field of nanotechnologies opens up many exciting opportunities and benefits for new materials with significantly improved properties as well as some revolutionary applications in the fields of energy, environment, medicine, etc.

These new materials potentially pave the way to considerable innovations in many industries of the 21st century strictly only if associated risks are perfectly under control for workers, consumers and the environment.

So, one can understand easily why the so-called Nanosafety is now considered as a specific new scientific area, gaining in importance and maturity each days.

Following the successful outcome of the two past **international conferences on Safe production and use of nanomaterials: Nanosafe 2008 and 2010**, the organizing committee has the pleasure to welcoming you again in Grenoble with some of the most famous specialists of the field in the world.

In addition of the standard issues addressed in previous Nanosafe conferences such as Toxicology, Ecotoxicology, Expology, Detection, Life Cycle Analysis, Regulation and Standardization, new topics of great interest will be dealt with this year, concerning Governance plus practical Risk Management for OSH experts and Societal issues.

In order to enhance the exchanges and conviviality three debates will be organized around Nano Governance, Toxicology and Ethics.

We hope that you will appreciate this new Nanosafe edition like the previous ones.

This is your Nanosafe conference, please enjoy.

The Nanosafe2012 organisers



François Tardif



Vanessa Gaultier



Frédéric Shuster

Monday 12/11/12		Tuesday 13/11/12		Wednesday 14/11/12		Thursday 15/11/12		
16:30-19:30 Registration	07:30. Registration 08:30 Welcome from the organizing committee Conference opening: F. Tardif Nanogovernance, trends and limitations Chairman: G. Katagariakakis F. Schuster- F. Roure - C. Engelman - D. Bernard - A. Grobe Auditorium		10:30-11:00: coffee break		08:00 Session 3 Toxicology limited to: nano-bio-interactions modelling, nanoparticles biotransformation and degradation in cells, tissues and environment, nanoparticles translocation across barriers G. Oberdorster Auditorium		08:00 Session 4 Environmental interactions of nanomaterials J. Rose Auditorium	
	11:00 Session 1 Exposure: assessment, modelling and case studies D. Brouwer Auditorium		11:45 SCAFFOLD Room C		08:45 Detection Room B		08:45 Environments Interactions Auditorium	
11:45 Exposure assessment Auditorium		11:45 Session 2 Detection and identification of engineered nanoparticles D. Pui Auditorium		10:00-10:30: coffee break		10:30 Session 5 Nanomaterials release from aging, mechanical constraints and solubilization, incineration, etc. T. Nguyen Auditorium		
13:00-14:00: lunch		14:00 Session 6 et 7 Secure industrial production and protection technology Luana Golemski Auditorium		10:30 Session 10 Life Cycle Analysis Commercial equipment Robert Blair Auditorium		10:30 Session 9 Ethics and societal issues A. Grimbaum Room C		
14:45 Detection and identification Auditorium		14:45 Exposure assessment Room B		11:15 Regulation and standardisation Auditorium		11:15 Nanomaterials - release Auditorium		
16:30-19:30 Registration		16:30 Session 6 et 7 Secure industrial production and protection technology Luana Golemski Auditorium		11:45 Toxicology Room B		11:15 Ethics and societal issues Room C		
17:15 Secure industrial production Auditorium		17:15 Exposure assessment Room B		12:30-13:30: lunch		12:30-13:30: lunch		
18:30-19:30 Panel discussion Governance F. Roure Auditorium		18:30-21:00 Poster exhibition/Evening event. Ballroom 1st floor		13:30 Session 8 Life cycle analysis, recycling, waste management and disposal B. Nowack Auditorium		13:30 Panel discussion The opinion of the civil society A. Grimbaum Room B		
				14:15 Life Cycle Analysis Auditorium		14:30 Toxicology Room B		
				14:30 Regulation and standardization Room C		14:30 Toxicology Room B		
				15:30-16:00: coffee break		15:30-16:00: coffee break		
				16:00-16:00 Toxicology Room B		16:00-17:00 Environmental interactions Auditorium		
				16:00-17:15 Life Cycle Analysis Room C		16:00-19:00 NANOHOUSE Room B		
				17:15 Secure industrial production Room C		17:00 -18:00 Toxicology Auditorium		
				20:00-23:30: cocktail party Transport by cable car to La Bastille		18:00-18:00 Nanomaterials - release Room C		
				Conclusions: V. Gaubier; F. Schuster; F. Tardif.		End of the conference: 18:15		
				Next NanoSafe Conference: November 2014				

PROGRAMME

Monday 12 November

16:30-19:30 Registration

Tuesday 13 November

MINATEC AUDITORIUM

8:15-8:50 Welcome by the Organizing Committee
François Tardif (*CEA, France*)

Conference opening

(Chair: Georgios Katalagarianakis)

PL0a Innovation perspectives through nanomaterials: the integrated approach at CEA
8:50-9:10 Frédéric Schuster (*CEA, France*)

PL0b Codes, Standards and Regulations in preparation at World-wide Level
9:10-9:30 Françoise Roure (*French Ministry of Finance, Economy and Industry, France*)

PL0c Regulation, risk and the global nanotechnology workplace
9:30-9:50 Cassandra Engeman (*UCSB, U.S.A*)

PL0d Are specific regulations for nanomaterials efficient?
9:50-10:10 Daniel Bernard (*Arkema, France*)

PL0e Innovation Governance: Challenges in the field of Nanotechnologies
10:10-10:30 Antje Grobe (*University of Stuttgart, Germany*)

10:30-11:00 Coffee-break

Session 1: Exposure assessment

(Chair: Derck Brouwer)

1a. Workplace and release studies

PL1 Engineered nanomaterials: from source to dose and the role of measurement
11:00-11:35 devices and measurement strategies
Derck Brouwer (*TNO Quality of Life, The Netherlands*)

O1a-1 Observatory of nanoaerosol release from electronic household products
11:45-12:00 Gwi-Nam Bae, S-H Park, S-B Lee (*Korea Institute of Science and Technology, Korea*)

O1a-2 Aerosol emission assessment during soldering process
12:00-12:15 Virginia Gómez, S. Irusta, F. Balas, J. Santamaría (*INA, Spain*)

O1a-3 Exposure to aerosols associated with cleanout operations of a reactor producing
12:15-12:30 nanocomposite thin films embedded with silver nanoparticles
Olivier Witschger, S. Bau, B. Bianchi, R. Wrobel, V. Matera (*INRS, France*)

O1a-4
12:30-12:45

13:00-14:00 Lunch

Session 2: Detection and identification of engineered nanoparticles*(Chair: David Y.H. Pui)***2a. Synthesis, aerosolization, and tracing of nanoparticles**

- PL2**
14:00-14:35 Detection and Identification: Instrumentation and Calibration for Air/Liquid/Surface-borne Engineered Nanoparticles
David Y.H. Pui (*Laboratory University of Minnesota, U.S.A*)
- O2a-1**
14:45-15:00 Towards the efficient and versatile syntheses of nanomaterials for nanosafety investigation
Aurélien Auger, V. Barthès, N. Wartenberg, S. de Sousa Nobre, L. Golanski, H. Perche, O. Poncelet, P. Capron (*CEA, France*)
- O2a-2**
15:00-15:15 Metal impurities provide useful tracers for identifying exposures to airborne single-walled carbon nanotube particles released from work-related processes
Pat E. Rasmussen, I. Jayawardene, H. David Gardner, M. Chénier, C. Levesque, J. Niu (*Health Canada, Canada*)
- O2a-3**
15:15-15:30 Strategies for radiolabelling of carbon nanoparticles
Stefan Schymura, I. Cydzik, A. Bulgheroni, F. Simonelli, U. Holzwarth, J. Kozempel, K. Franke, N. Gibson (*HZDR, Institute of Resource Ecology, Germany*)
- O2a-4**
15:30-15:45 Generation and characterization of dry nanopowders and carbon nanotubes using a shaker-atomizer type disperser
Shi-Nian Uang, S-M. Hung, C-J. Tsai (*Institute of Occupational Safety and Health, Taiwan*)
- O2a-5**
15:45-16:00 Carbon nanotubes suspension study by vortex shaker: sampling and mass analysis
Aurélien Ustache, O. Le Bihan, D. Bernard, E. Peyret, O. Aguerre-Chariol (*INERIS, France*)
- 16:00-16:30 Coffee-break

Session 6/7: Secure industrial production and protection technology

(Chair: Luana Golanski)

6a. Secure industrial production

- PL6**
16:30-17:05
Developments in nano protection
Luana Golanski, A. Guiot, S. Motellier, S. Clavaguera, C. Brouard, N. Wartenberg, H. Perche, F. Tardif, P. Capron, S. Artous, C. Durand, V. Mossuz, C. Desvergnès, M. Dubosson (CEA, France)
- O6a-1**
17:15-17:30
Towards large scale aligned carbon nanotube composites: an industrial safe-by-design and sustainable approach
Pascal Boulanger, L. Belkadi, M. Pinault, J. Descarpentries, M.P. N. Ghem, C. Reynaud, M. Mayne-L'Hermite (CEA, France)
- O6a-2**
17:30-17:45
Detection and identification of airborne multi-walled carbon nanotubes in arc discharge production
Christina Isaxon, L. Ludvigsson, P. Nilsson, M. Hedmer, H. Tinnerberg, Maria E. Messing, J. Rissler, V. Skaug, M. Bohgard, J. Pagels (Lund University, Sweden)
- O6a-3**
17:45-18:00
Plasma polymerized coating as a protective layer of carbon nanotubes grafted on carbon fibers
Antinéa Einig, P. Rumeau, J. Maguin, Y. Magga, S. Desrousseaux, J. Bai (Ecole Centrale Paris, France)
- O6a-4**
18:00-18:15
Silica-coating as protective shell for the risk management of nanoparticles
Davide Gardini, M. Blosi, C. Delpivo, S. Ortelli, A. Costa (CNR-ISTEC, Italy)
- O6a-5**
18:15-18:30
Pilot-scale platform for nanopowder synthesis by laser/plasma hybrid processes
Yann Leconte, A. Quinsac, D. Porterat, O. Sublemontier, N. Herlin-Boime, C. Reynaud, J-P. Dufour, L. Boufendi, F. Schuster (CEA, France)

Panel discussion: Governance

(Moderator: Françoise Roure)

- 18:30-19:30
Governance: what will change in the near future regarding the different actors: managers, workers and safety managers?
Françoise Roure (French Department of finance, economy and Industry, France), Elvio Mantovani (AIRI/Nanotec IT, Italy), Antje Grobe (University of Stuttgart, Germany)
- 18:30-21:00
Poster Exhibition/Evening event

ROOM B**Session 1: Exposure assessment***(Co-chair: Catherine Durand)***1b. Methodology**

- O1b-1** 14:45-15:00 A modular tool for analyzing cascade impactors data to improve exposure assessment to airborne nanomaterials
Sébastien Bau, O. Witschger (*INRS, France*)
- O1b-2** 15:00-15:15 Exploratory study on two statistical methods to analyse time resolved data obtained during nanomaterial exposure measurements
Frédéric Clerc, G.H. Njiki-Menga, O. Witschger (*INRS, France*)
- O1b-3** 15:15-15:30 Assessment of nanoparticle agglomeration energy via rheological routes: a key parameter for control banding exposure assessment
François Henry, J. Bouillard, A. Vignes, O. Dufaud, L. Perrin, P. Marchal (*INERIS, France*)
- O1b-4** 15:30-15:45 Approach to the exposure assessment of MWCNT by considering size distribution and oxidation temperature of elemental carbon
Mariko Ono-Ogasawara, M. Takaya, H. Kubota, Y. Shinohara, S. Koda, E. Akiba, S. Tsuruoka, T. Myojo (*Japan national institute of occupational safety and health, Japan*)
- O1b-5** 15:45-16:00 Is exhaled breath condensate representative of deep lung and suitable for exposure assessment to nanoparticles?
Muriel Dubosson, C. Desvergne, V. Mossuz, M. Cottier J. Pourchez, J-M. Vergnon (*CEA, France*)
- 16:00-16:30 Coffee-break

Session 1: Exposure assessment*(Co-chair: Olivier Witschger)***1c. Tools and non-occupational exposure**

- O1c-1** 17:15-17:30 Pro et con analysis of occupational exposure assessment tools and concepts for nanomaterials
Biase Liguori, S. F. Hansen, K. Alstrup Jensen, A. Baun (*Technical University of Denmark, Denmark*)
- O1c-2** 17:30-17:45 Urban air pollution of Ostrava region by nanoparticles
Zdeňka Kaličáková, P. Danihelka, K. Lach, V. Míčka. (*VŠB–Technical University of Ostrava, Institute of Public Health Ostrava, Czech Republic*)
- O1c-3** 17:45-18:00 Nanoproduct simplified direct exposure assessment toolkit
Yves Sicard, F. Tardif, J. Vendel (*CEA, France*)
- O5c-8** 18:00-18:15 Exploring release and recovery of nanoparticles from polymer nanocomposites using commercial polyamide-based nanocomposites as a model

Tuesday 13 November 2012

ROOM B

Martí Busquets-Fité, R. Zanasca, C. Citterio, L. Mercante, E. Fernández ,
Gemma J. Socorro Vázquez-Campos, V. Puentes (*ICN, Spain*)

18:30-21:00 Poster exhibition/ Evening event

ROOM C**Session 13: Satellite meeting****13b. TRIMATEC***(Chair: Claudine Colin)*

- O13b-1** 14:00-14:20 Trimatec competitiveness cluster: management of controlled environment for nano fields
Claudine Colin (*TRIMATEC, France*)
- O13b-2** 14:20-14:40 Treatment of liquid effluents containing nanomaterial
Jérôme Labille, J-Y. Bottero, J. Rose (*CEREGE, France*)
- O13b-3** 14:40-15:00 Safe ecodesign and sustainable research Applied to Nanomaterial Development (SERENADE consortium)
Jean-Yves Bottero, J. Rose, A. Masion (*CEREGE, France*)
- O13b-4** 15:00-15:20 Synthesis of active TiO₂ nanopowders and thin layers using supercritical carbon dioxide processes - Application to effluent treatment
Audrey Hertz, J. Cruz, L. Schrive, Y. Barre, F. Charton (*CEA, France*)
- O13b-5** 15:20-15:40 Examples of system engineering for nanoparticles handling and studies.
Pierre Bombardier (*FAURE QEI, France*)
- O13b-6** 15:40-16:00 Personal Protective Equipment against Nanoparticle-Selection evaluation and future standard to be built.
Samuel Ozil (*Honeywell Protective Clothing, France*)
- 16:00-16:30 Coffee-break

Session 2: Detection*(Co-chair: Charles Motzkus)***2b. Nanoparticles for biological application**

- O2b-1** 17:15-17:30 Study of Nanoparticles localization in organs and tissues of rats after intraperitoneal and intragastrical administration
Boris B. Dzantiev, S.G. Klochkov, O. D. Hendrickson, A. V. Zherdev, S.O. Bachurin (*A.N. Bach Institute of Biochemistry, Russia*)
- O2b-2** 17:30-17:45 Risk Assessment of Released cellulose nanocrystals–mimicking inhalatory exposure
Carola Endes, S. Mueller, O. Schmid, D. Vanhecke, S. Camarero Espinosa, E. Johan Foster, A. Petri-Fink, B. Rothen-Rutishauser, C. Weder, Martin J.D. Clift (*Adolphe Merkle Institute/University of Fribourg, Switzerland*)
- O2b-3** Colloidal stable, polymer encapsulated quantum dots for biological applications

- 17:45-18:00 Isaac Ojea-Jimenez, J. Piella, P. Mulvaney, V. F. Puntès (*Catalan Institute of Nanotechnology, Spain*)
- O2b-4**
18:00-18:15 Membrane model as a new methodology for nanotoxicology Investigation
Juliana Cancino, T. M. Uemura, Paula Lins, P. B. Miranda, V. Zucolotto (*University of São Paulo, Brazil*)
- O2b-5**
18:15-18:30 Engineered nanomaterial quantification in complex matrices: PIXE case studies
Omar Lozano, J. Mejia, J. Laloy, O. Toussaint, J. Dogné, S. Lucas (*Research Centre for the Physics of Matter and Radiation, Belgium*)
- 18:30-21:00 Poster exhibition/Evening event

Posters session: 18:30-21:00

- P1b-1** Occupational exposure assessment facility at Vito
Evelien Frijns, I. Nelissen, P. Berghmans (*Vito nv, Belgium*)
- P1b-2** Risk assessment of nanomaterials
Javad Malakootikhah, A. A. Razaghi (*University of Tehran, Iran*)
- P1c-1** Application of current available models for the exposure assessment of nanoparticles from consumer products
Anja Köth, A.Luch, and M. Enrico Götz (*Federal institute for risk assessment, Germany*)
- P1c-2** Development of a nano exposure and contextual information database (NECID)
Wouter Fransman, J. Pelzer, W. Stoppelmann, D. Brouwer, I. Koponen, D. Bard, O. Witschger, A. Zugasti Makazaga, E. Jankowska, T. Kanerva, M. Berges (*TNO, The Netherlands*)
- P2a-1** Effective and selective extraction of silver nanoparticles from environmental water by an ionic exchange resin
Lingxiangyu Li, K. Leopold, M. Schuster (*Technische universität München, Germany*)
- P2a-2**
- P2a-3** Application of enzyme immunoassay for fullerene C₆₀ detection in rats' organs
Olga d. Hendrickson, N. Fedyunina, A. Zherdev, P.Sveshnikov, B. Dzantiev (*Russian Academy of Sciences, Russia*)
- P2a-4** New perspectives in manufactured nanoparticles characterisation: over the size limits
Gaëtane Lespes, C. Henault (*University of Pau, France*)
- P2a-5** Quantitation of carbon nanotubes on filters using a laser-induced breakdown spectroscopy system. Laboratory and onsite measurements
J.-B.Sirven, C. Quéré, S. Motellier, A. Guiot, F. Gensdarmes (*CEA, France*)
- P2a-6** Differentiated anthropogenic ambient particle size spectra with a new U-SMPS set-up
Jürgen Spielvogel, M. Weiss (*Ppalas[®] gmbh, Germany*)
- P2a-7**
- P2a-8** Particle sampling by TEM grid filtration
O.L.C. Le bihan, B. R'Mili, C. Dutouquet, O. Aguerre-Charriol, E. Frejafon (*INERIS, France*)

- P2a-9** Aerosol size distribution estimation and associated uncertainty for measurement with a SMPS
Loic Coquelin, N. Fischer, T. Mace, C. Motzkus, F. Gensdarmes, G. Fleury, L. Le Brusquet (*INE, France*)
- P2a-10** Radiolabelling of engineered silver and titania nanoparticles as a tool for sensitive detection of nanoparticle release from surface coatings
Heike Hildebrand, K. Franke, N. Gibson, I. Cydzik, F. Simonelli, A. Bulgheroni, U. Holzwarth, E. Bilz, A. Freyer (*Helmholtz-Zentrum, Germany*)
- P2a-11** Comparison of methodologies to measure number based size distribution on polydisperse nanoparticles
Samuel Legros, V. Barthes, A. Guiot, S. Motellier, P. Capron, L. Golanski (*CEA, France*)
- P2a-12** Non destructive and fast method for the detection of superparamagnetic iron oxide nanoparticles (SPION) biodistribution based on their magnetic properties
Lionel Maurizi, V. Bernau, U. Sakulkhu, A. Gramoun, G. Coullerez, H. Hofmann (*Ecole polytechnique fédérale de Lausanne, Switzerland*)
- P3a-1** Silver doped hydroxyapatite composites for long-term bone TERAPHIA
Olena Ivashchenko, I. Uvarova, N. Ulianchych (*National Academy of Sciences of Ukraine, Ukraine*)
- P3a-2** Cytotoxic effects of polyethylcyanoacrylate/chitosan nanoparticles
Brenda Cecilia Gasca Zacarías, R. Díaz Torres, P. Ramírez Noguera (*National University of Mexico, México*)
- P3a-3** Reactivity of nanoaluminum in physiological solutions
Karepina E.E., G.A.Yu (*Tomsk Polytechnic University, Russia*)
- P3a-4** Determination of SPIONS nanoparticles biosafety: problems of interferences with cytotoxic assays
Lyes Tabet, L. Barhoumi, M. Welman, L. Ben Taher, L. Smiri, H. Abdelmelek, K. Maghni (*Research Center HSCM, Université de Montréal, Canada*)
- P3a-5** Renal metallothionein expression by nano zinc particles in cadmium-treated rats
Mohammad Kazem Koohi, F. Asadi, M. Abas Ali Pour Kabire, G. Sadeghi Hashtjin, M. Hejazy (*University of Tehran, Iran*)
- P3a-6** Differences in nanotoxicity responses between human bronchial smooth muscle cells (HBSMC) of normal and asthmatic subjects following exposure to quantum dots (QDS) nanoparticles
Lyes Tabet, M. Welman, L. Castellanos, K. Maghni. (*Sacre-Coeur Hospital of Montreal, Canada*)
- P3a-7** Comparative evaluation of cytotoxicity and DNA damage induction by carbon nanotubes, cerium dioxide, titanium dioxide and silver nanoparticles in mammalian cells
Leonardo Pereira Franchi, T. AJ Souza, E. Y Matsubara, J M Rosolen, C. Satie Takahashi, CS (*Department of Genetics, FMRP-USP, Brazil*)

- P3a-8** Genotoxicity in RTG-2 fish cell line upon exposure to different dimensions of silver nanoparticles detected by the comet assay
Mahmoud Ghobadi, H. Farahmand, A. Mirjalili (*University of Tehran, Iran*)
- P3a-9** Two “faces” of carbon nanotubes
Aneta Fraczek-Szczypta, E. Menaszek, S. Blazewicz (*University of Science and Technology, Poland*)
- P3a-10** Study of biocompatible and hemocompatible properties of amorphous hydrogenated carbon coatings produced by pulsed magnetron discharge.
Julie Laloy, C. Lopez-Garcia, J. Colaux, F. Mullier, M. Fransolet, C. Michiels, JM Dogné & S. Lucas (*Department of Pharmacy, NAMEDIC, NTHC, Belgium*)
- P3a-11** Effect of nano-filler particles and methacrylate monomers, separate and in combination, on the secretion of cytokines
V Ansteinsson, J.T. Samuelsen, J.E. Dahl and N.R. Gjerdet (*University of Bergen, Norway*)
- P3a-12** *In vivo* genotoxicity of titanium dioxide nanoparticle
Charlène Relier, F. Robidel, B. Trouiller (*INERIS, France*)
- P3a-13** Insights into titanium dioxide nanoparticle and fine genotoxicity in human lymphocytes
Andrea Zijno, C. Andreoli, F. Barone, P. Degan, B. De Berardis, G. Leter, R. Crebelli (*Istituto Superiore di Sanità, Roma*)
- P3a-14** Highly concentrated silica nanoparticles affect the activities of neural stem cell line
Kouki Fujioka, S. Hanada, Y. Inoue, F. Kanaya, K. Shiraishi, Y. Manome (*The Jikei University School of Medicine, Japan*)
- P3a-15** Toxicological influence of giving the silica nanoparticles on cultured central nerves cells
Yuriko Inoue, K. Fujioka, S. Hanada, F. Kanaya, K. Shiraishi, Y. Manome, M. Takayanagi (*Toho University, Japan*)
- P3a-16** Effect of surface modification on Zn ions and ROS production of ZnO nanoparticles
Mu Yao Guo, Y. Hang Leung, Alan M. C. Ng, Fang Zhou Liu, Yip Hang Ng, Aleksandra B. Djurišić, Wai Kin Chan (*The University of Hong Kong, Hong Kong*)
- P3a-17** Cytotoxicity and genotoxicity of silver nanoparticles with different sizes in mammalian cells
Tiago A. Jorge de Souza, L. Pereira Franchi and C. Satie Takahashi, (*Department of Genetics, FMRP-USP, Brazil*)
- P3a-18** *In vivo* toxicity of enoxaparin encapsulated in mucoadhesive nanoparticles: topical application in a wound healing model
S.C Huber, P.D. Marcato, N. Durán, J.M Annichino-Bizzacchi (*Unicamp, Campinas, Brazil*)
- P3a-19** Phytotoxic and genotoxic effects of PVP coated ZnO nanoparticles on garlic

(*allium sativum* L.) root tip cells
Changshan Xu, K. Eit, M. Wang, Y. Li, B. Sun,
(*Centre for Advanced Optoelectronic Functional Materials Research, Northeast Normal University, P. R. China*)

- P3a-20** Internalisation of aluminium oxide nanoparticles into human cells: impact of particle size on the quantitative uptake
Steffi Böhme, H-J. Stärk, T. Meissner, D. Kühnel, W. Busch (*UFZ - Helmholtz-Centre for Environmental Research, Germany*)
- P3a-21** Immunological assays as an opportunity of assessment of health risks of airborne particle mixture including nanoparticles
Táňa Brzicová, I. Lochman, P. Danihelka, A. Lochmanová, K. Lach, V. Mička (*VŠB –Faculty of Safety Engineering, Czech Republic*)
- P3a-22** Altered characteristics of differently functionalized silica nanoparticles in various environments with possible implications for biological impacts
Emilia Izak, M. Voetz, S. Eiden, A. Duschl and V.F. Puentes (*Bayer Technology Services GmbH, Germany*)
- P3a-23** Enhanced antiproliferation of cancer cells by biocompatible multifunctional microbial exopolysaccharide stabilized magnetic nanoparticles
Balasubramanian Sivakumar, R. Girija Aswathy, R. Sreejith, Y. Nagaoka, M. Suzuki, Y. Yoshida, T. Maekawa, D. Nair Sakthikumar (*Bio Nano Electronics Research Center Graduate School of Interdisciplinary New Science Toyo University, Japan*)
- P3a-24** Copper(ii) oxide nanoparticles penetrate into HEPG2 CELLS, exert cytotoxicity via oxidative stress and induce proinflammatory response
Jean-Pascal Piret, D. Jacques, J.-N. Audinot, J. Mejia, E. Boilan, F. Noël, M. Fransolet, C. Demazy, S. Lucas, C. Saout and O. Toussaint (*URBC, Namur Nanosafety Center (NNC), Belgium*)
- P3a-25** Electrical measurement of the interactions between gold nanoparticles and biological membrane
Young-Rok Kim, M-C. Lim, K-P. Lee (*Department of Food Science and Biotechnology, Kyung Hee University, Republic of Korea*)
- P3a-26** Metal homeostasis interferences in hepatocytes cells treated by CuO nanoparticles
Martine Cuillel, M. Chevallet, C. Fauquant, P. Charbonnier, D. Cassio, I. Pignot-Paintrand, E. Mintz and I. Michaud-Soret (*CNRS / CEA, France*)
- P3a-27** Transnational access at VITO through the Fp7 nano research infrastructure
Evelien Frijns, I. Mertens, K. Tirez, G. Vanermen, S. Voorspoels, R. Persoons, S. Mullens, I. Nelissen (*VITO NV, Belgium*)
- P3a-28** Source, activity and toxicity of nanomaterials for biological systems
Ghassem Amoabediny, J. Malakootikhah, I. Alahdadi, F. Yazdian (*University of Tehran, Iran*)
- P3a-29** TiO₂ nanoparticles and bulk material stimulate human peripheral blood mononuclear cells
Dietmar Fuchs, S. Schroecksnadel, N. Herlin, M. Carriere,

(Biocenter, Innsbruck Medical University, Austria)

- P3a-30** In vitro testing of nanoparticles dissolution
Anna Godymchuk, E. Yunda (*Tomsk Polytechnic University, Russian Federation*)
- P3a-31** Particulate cobalt toxicity and internalization in lung cells
Véronique Malard, C. Darolles, N. Sage, P. Guéraud, M. Floriani, J. Armengaud (*CEA, IRSN, France*)
- P3a-32** Effects of physicochemical properties, exposure doses and cell types on cytotoxicity of zinc oxide nanoparticles
Yan (Mary) Zhang, Kathy C. Nguyen, D. E. Lefebvre, P. S. Shwed, J. Crosthwait, G. S. Bondy, Azam F. Tayabali (*Environmental Health Sciences and Research Bureau, Health Canada, Canada*)
- P3a-33** Proteomic study of the molecular responses of mouse macrophages to copper oxide nanoparticles
Thierry Rabilloud, S. Triboulet, C. Aude-Garcia, M. Carrière, H. Diemer, F. Proamer, A. Habert, M. Chevallet, V. Collin-Faure, D. Hanau, A. Van Dorselaer, N. Herlin-Boime (*CNRS, France*)
- P3a-34** Toxicological evaluation of TiC nanoparticles orally administered in a rat model
Julie Laloy, O. Lozano, L. Alpan, J. Mejia, O. Toussaint, J-M. Dogn, S. Lucas (*NNC, NARILIS, University of Namur, NAMEDIC Belgium*)
- P3a-35** *In vitro* toxicity assessment of gold nanoparticles in biological media
Sadequa Sultana, N. Djaker, M. Salerno, S. Boca, S. Astilean, H. Hlawaty, M.Lamy De La Chapelle (*Université Paris 13, France*)
- P3a-36** Comparison cytotoxic potency of zinc oxide nanoparticles on five cellular lines.
Lidia Zapór, M. Szewczyńska (*Central Institute for Labour Protection – National Research Institute, Poland*)
- P3a-37** Respiratory effects of repeated instillations of iron, manganese, and chromium oxides nanoparticles in mice
Mirlande Prémumé, A. Simon-Deckers, G. Beaune, O. Durupthy, J. Boczkowski, S. Lanone, (*INSERM, France*)
- P3a-38** Morphological and cytohistochemical evaluation of renal effects of cadmium-doped silica nanoparticles given intratracheally to rat.
Teresa Coccini, E. Roda, S. Barni, L. Manzo (*Toxicology Division, Salvatore Maugeri Foundation IRCCS, and European Centre for Nanomedicine, University of Pavia, Italy*)
- P3a-39** Titanium oxide nanoparticles toxicity causes functionality and DNA damage in buffalo (*bubalus bubalis*) sperm *in vitro*.
Gautam Kaul and Kamlesh Pawar (*Biochemistry Department, National Dairy Research Institute, Government of India Lab., India*)
- P3a-40** Cytotoxicity and genotoxicity of zinc oxide nanoparticles in human neuroblastoma cells
Carla Costa, V. Valdiglesias, G. Kılıç, B. Laffon, J. P. Teixeira (*Portuguese*)

National Institute of Health, Portugal)

- P3a-41** Innovative nano-qsar technology for early detection of nanoparticles' toxicity
Natalia Novoselska, V. Kuzmin, A. Artemenko (*I.I.Mechnikov Odessa National University, Ukraine*)
- P3a-42** In vitro toxicity of carbon nanotubes: impact of acid functionalization
Agathe Figarol, J. Pourchez, D. Boudard, D. Bernache-Assolant, M. Cottier, and P. Grosseau (*Ecole Nationale Supérieure des Mines, LINA, France*)
- P3a-43** Role of metal oxide nanoparticle in welder's lung injury
A. Simon-Deckers, P. Andujar, B. Fayard, B. Clin, J. Boczkowski, J-C. Pairon, J. Doucet, F. Gallateau-Sallé, S. Lanone (*INSERM, CNRS, France*)
- P3a-44** MTT and LDH interlaboratory assays for assessing in vitro cytotoxicity of engineered nanomaterials
C. Costa, J- P. Teixeira, A. Dhawan, A. Pandey, B. Laffon, J. Fernandez Tajés, V. Valdiglesias, D. Fuchs, S. Schroecksnadel, M. Carrière, S. Bonassi, A. Basak Engin, E. Coskun, B. Karahalil, Nathalie Herlin-Boime
(*Environmental Health Dept., National Institute of Health Dr. Ricardo Jorge, Porto, Portugal*)
- P3a-45** Understanding the impact of np physicochemical properties in biological fluid and their resultant cellular interaction *in vitro*
Vera Hirsch, C. Kinnear, B. Rothen-Rutishauser, Martin J. D. Clift, A. Petri-Fink
(*Adolphe Merkle Institute, University of Fribourg, Switzerland*)
- P3a-46** Biocompatibility evaluation of medical devices incorporating nanomaterials
Sylvie Framery (*NAMSA Europe, France*)
- P3a-47** Genotoxicity and proinflammatory effects of nanosilica via oral route: *in vitro* and *in vivo* approaches
Adeline Tarantini, K. Hogeveen, S. Huet, G. Jarry, R. Lanceleur, L. Le Hegarat, A. Mourot, M. Poul, J-G. Rolland, V. Fessard. (*ANSES, France*)
- P3a-48** In vitro evaluation of cellular response induced by ZnO nanoparticles, zinc ions and non-nano ZnO in fish cells
Mar Babin, C. del Rio, José L. Pareja, C. García-Gómez, D. Fernández (*INIA. Department of Environment, Spain*)
- P3a-49** Genotoxicity of cerium dioxide nanoparticles (CeO₂ nps) on mice oocyte.
Raphaël Rollais, M. Auffan, J. Perrin, V. Tassistro, T. Orsière, A. Botta, J. Rose, B. Courbiere (*Institut Méditerranéen de Biodiversité et d'Ecologie, Faculté de Médecine de l'Université d'Aix-Marseille, France*)
- P3a-50** Effects of amorphous silica nanoparticles on human alveolar epithelial cells
Mathilde Delaval, R. Guadagnini, S. Vranic, F. Marano, A. Baeza-Squiban, S. Boland (*Sorbonne Paris Cité, CNRS France*).

- P3a-51** How do carbide enm dispersions evolve in an *in vitro* assessment?
Jorge Mejia, O. Lozano, J-P. Piret, D. Jacques, C. Saout, J.M. Dogné, O. Toussaint, S. Lucas, (*Research Centre for the Physics of Matter and Radiation, Laboratory of Biochemistry and Cellular Biology (URBC), Belgium*)
- P3a-52** Stability of SiC and TiC nanoparticles during *in vitro* assessment
Jorge Mejia, V. Valembos, J-P. Piret, C. Saout, Jean-Michel Dogné, Olivier Toussaint, Stéphane Lucas (*Research Centre for the Physics of Matter and Radiation, Laboratory of Biochemistry and Cellular Belgium*)
- P3a-53** Cytotoxicity of MWCNT and SiO₂ nanoparticles at different stages of their life cycle as nanocomposite fillers
Ezequiel Mas del Molino, Gemma Vilar, G. Janer, E. Fernández-Rosas, S. Vázquez-Campos (*LEITAT Technological Center, SPAIN*)
- P3a-54=>
(03b-2)** Nitric oxide releasing-iron oxide magnetic nanoparticles for biomedical applications: cell viability, apoptosis and cell death evaluations
Renata de Lima, J. Luiz Oliveira, A. Ludescher, M. A. M. Molina, R. Itri, Amedea B. Seabra, Paula Haddad (*Departamento de Biotecnologia, Universidade de Sorocaba, Brazil*)
- P3a-55** Cytotoxicity and genotoxicity of biogenic silver nanoparticles
R. De Lima, D. Ballottin, Priscyla D. Marcato, L. Tasic, Nelson Durán (*Osasco University, Brazil*)
- P3a-56** Biocompatibility and cytotoxicity study of nanophotonic contact lens material
Marija Tomic, J. Muncan, D. Stamenkovic, M. Jokanović, L. Matija (*Faculty of Mechanical Engineering, University of Belgrade, Serbia*)
- P3a-57** Nanoparticles in paints; a new strategy to protect façades and surfaces?
Jean-Pierre Kaiser, L. Diener and P. Wick (*EMPA, Switzerland*)
- P3a-58** Pulmonary toxicity after inhalation of silicon carbide nanoparticles in rat.
Julie Laloy, O. Lozano, L. Alpan, O. Toussaint, B. Masereel, J-M. Dogné & S.Lucas (*NNC, NARILIS, University of Namur, NAMEDIC, NTHC Belgium*)
- P3a-59** Development and validation of a whole-body inhalation exposure model for the exposition of rats to nanoparticles aerosol
Julie Laloy, O. Lozano, L. Alpan, O.Toussaint, S. Rolin, B. Masereel & S. Lucas (*NNC, NARILIS, University of Namur, NAMEDIC, NTHC Belgium*)
- P3a-60** Functionalized double walled carbon nanotubes (DWCNTS) for targeted drug release
T. Somanathan, N. Gokulakrishnan, (*Vels University, Chennai, India*)
- P3a-61** Biodistribution studies on nanoparticles are so far of limited use for PBPK modeling
Gunnar Johanson, U. Carlander (*Karolinska Institutet, Sweden*)
- P3a-62** Toxicokinetics of zinc oxide nanoparticles in rats
Soo-Jin Choi, Hae-Eun Chung, Jin Yu, Miri Baek, Jeong-A Lee, Min-Seok Kim, Su-Hyon Kim, Eun-Ho Maeng (*Department of Food Science and Technology, Seoul Women's University, South Korea*)

- P4a-1** Evaluation of toxicity of the nanoscale materials for mammals and environment
Rostyslav Stoika, N. Boyko, Y. Senkiv, Y. Shlyakhtina, R. Panchuk, R. Bilyy, Y. Filyak, Y. Kit, N. Skorohyd, O. Klyuchivska, H. Falfushinska, L. Gnatyshyna, O. Stoliar, A. Zaichenko, N. Mitina, A. Ryabceva.
(*Institute of Cell Biology, Ukraine*)
- P4a-2** Metal oxide nanoparticle transport in porous media – an analysis about (un)certainities in environmental research
Ilona Heidmann (*University of Koblenz-Landau, Germany*)
- P4a-3** Assessing the heteroaggregation of manufactured nanoparticles with naturally occurring colloids in a typical surface water
Jérôme Labille, A. Praetorius, C. Harns, J-Y. Bottero, J. Brant, M. Scheringer
(*Aix-Marseille Université, CNRS, IRD, CEREGE, France*)
- P4a-4** Potential safety implications of nanoformulation of agrochemicals in crops production
Haixin Cui, X. Zhao (*The Chinese Academy of Agricultural Sciences, China*)
- P4b-1** Environmental dissemination of silver nanoparticles: which impact on crops?
Camille Larue, L. Cécillon, H. Castillo-Michel, S. Sophie, J. Bourguignon, M. Carrière, S. Bureau, V. Magnin, G. Sarret (*Isterre, UMR 5275, CNRS and Univ. J. Fourier, France*)
- P4b-2** Bio-interactions between proteins and CeO₂-nps with similar hydrodynamic radius
Françoise Rollin-Genetet, E. Artells, C. Seidel, W. Liu, J. Rose, A. Thiéry, C. Vidaud (*CEA, France*)
- P4b-3** Carbon nanotubes enhanced the lead toxicity on the freshwater fish
Diego Stéfani T. Martinez, Oswaldo L. Alves and E. Barbieri (*University of Campinas – UNICAMP, Brazil*)
- P4b-4** Colloidal stability of modified carbon nanotubes
Stefan Schymura, K. Franke (*HZDR, Institute of Resource Ecology, Germany*)
- P4c-1** Behavioural responses of *hediste diversicolor* (polycheta) to Ag, CdS, or CuO nanoparticles using the multispecies freshwater biomonitor[®] (MFB)
Hanane Perrein-Ettajani, P. Emmanuel Buffet, P. Wu, Douglas Gilliland, P. Reip, E. Valsami-Jones, C. Mouneyrac (*LUNAM université, UCO, MMS, France*)
- P4c-2** Combined effect of ZnO nanoparticles on zebrafish early development
Eugene Krysanov, T. Demidova (*Russian Academy of Sciences, Russia*)
- P4c-3** Effect of metal oxide nanoparticles on embryotoxicity of doxorubicin
Tatiana Demidova, E. Krysanov (*Russian Academy of Sciences, Russia*)
- P4c-4** Evaluation of multi walled carbon nanotubes ecotoxicology using standardized procedures on aquatic organisms

Florence Mouchet, C. Gancet, E. Pinelli, A. Perrault, F. Bourdiol, E. Flahaut, L. Gauthier, J-C. Boutonnet (CNRS UPS INPT, NAUTILE, Laboratoires ecolab/CIRIMAT/GRL, France)

- P4c-5** Adverse effect of surface modified nanoparticles on the aquatic environment
Younjung Jung, J-Sung Ra, and S. Kim (KIST-Europe, Campus E, Germany)
- P4c-6**
- P4c-7** Acute aquatic toxicity of photoactive TiO₂ nanoparticles anchored on kaolinite matrix to freshwater green algae
Kristina Čabanová, P. Peikertová, V. Matějka, J.Kukutschová (VŠB – Technical University of Ostrava, Czech Republic)
- P4c-8** Monitoring technique for the acute effect of TiO₂ nanoparticles from the behaviour of rice fish *orizias latipes* in vivo.
Masaru Irie, K. Kosuge, K. Shida, M. Kubo-Irie, K. Takeda (Waseda Univ, Japan)
- P4c-9** Antifungal and antibacterial activities of silver nanoparticles
Mohd Faiyaz Anwar, D. Yadav, R. Arora, J. Chandara, M. Samim (Department of Chemistry, Jamia Hamdard University, India)
- P4c-10 (04a-3)** Adsorption of organic pollutants to aqueous suspensions of carbon nanomaterials
Berit Glomstad, A. Booth, B. M. Jenssen, L. Sørensen, J. Liu, M. Shen (Norwegian University of Science and Technology, Norway)
- P5a-1** Carbon nanotubes sorbents and their potential risks to aquatic organisms
Daniela Plachá, A. Matlochová, K. Čabanová, P. Peikertová (VŠB-Technical University of Ostrava, Czech Republic)
- P5a-2** Ecologically friendly intermatrix synthesis of polymer stabilized silver nanocomposites: dealing with nanomaterial release.
Julio Bastos-Arrieta, M. Muñoz, Dmitri N Muraviev, P. Ruiz (Autonomous University of Barcelona, Spain)
- P5a-3** Characterization of nanoparticulate emissions from the incineration of wastes containing manufactured nanomaterials
Dinh-Trinh Tran, D. Fleury, D. Venditti, S. Durecu, A. Joubert, G. Ounoughene, E. Fiani, T. Meunier, O. Le Bihan, L. Le coq (INERIS, Ecole des mines de Nantes, France)
- P5a-4** Investigation of nanoparticle release from UV-curable polymeric nanocomposites
A.Freyer, E. Bilz, H. Hildebrand, K. Franke, R. Mehnert, E. Mai, (Leibniz Institute for Surface Modification, Germany)
- P5a-5** Characterization of released particles during abrasion test of nano-charged construction materials
C. Bressot, O. Aguerre-Chariol, A. Plassais, F. Rousseau, C. Haehnel, O. Le Bihan (INERIS, France)

- P5a-6** Release of nanomaterials from paint waste
Stefano Zuin, M. Gaiani, A. Ferrari, L. Golanski, F. Tardif (*Venice Research Consortium, Italy*)
- P5a-7** Potential release of carbon nanotubes from cnt composites during grinding process
Isamu Ogura, M. Kotake, M. Shigeta, M. Uejima, K. Saito, N. Hashimoto, A. Kishimoto, (*National institute of advanced industrial science and technology (AIST) technology research association for single wall carbon nanotubes, Japan*)
- P5a-8** Nanomaterials for controlled drug delivery system
T. Somanthan (*Vels University, india*)
- P6a-1** Release characteristics of single-wall carbon nanotubes during manufacturing and handling
Isamu Ogura, M. Kotake, Nao Hashimoto, K. Gotoh, A. Kishimoto, (*National Institute of Advanced Industrial Science and Technology (AIST), Technology Research Association for Single Wall Carbon Nanotubes (TASC), Japan*)
- P6a-2** Towards a nanosecured platform to assess risks along the industrial lifecycle of nanomaterials
Dominique Fleury, E. Frejafon, B. Debray, O. Aguerre-Chariol, A. Vignes and J. Bouillard (*INERIS, France*)
- P6a-3** Mapping the use of nanoparticles in Quebec's industries and research laboratories
Claude Emond, C. Ostiguy, I. Dossa, Y. Malki, C. Boily, D. Roughley, A. Plavski et C-A. Endo (*University of Montreal, Department of Environmental and Occupational Health Department, Canada*)
- P8a-1** Life cycle and destination of silver nanoparticles in environment
Ghassem Amoabediny, I. Alahdadi, J. Malakootikhah (*University of Tehran, Iran*)
- P9a-1** Towards nanoresponsibility
Dorothee Benoit Browaeys, Jean-Jacques Perrier (*VivAgora, France*)
- P9a-2** Nanosafety - risk governance of manufactured nanoparticles -- challenges of substance regulation under scientific uncertainty
Stefanie B. Seitz, J. Jahnel, and T. Fleischer (*Karlsruhe Institute of Technology (KIT), Germany*)
- PO** The ERA-Net SIINN Project: Safe Implementation of Innovative Nanoscience and Nanotechnology.
Rainer Hagenbeck, B. Fillon, D. Duret, L. Golanski, S. Legros (*Forschungszentrum Jülich GmbH, Germany/CEA, France*)
- P1** Innovative strategies, methods and tools for occupational risks management of manufactured nanomaterials (MNMs) in the construction industry
Jesús M. López de Ipiña (*TECNALIA Research & Innovation*) on behalf of the Scaffold Consortium

Wednesday 14 November

MINATEC AUDITORIUM

Session 3: Toxicology

(Chair: Günter Oberdörster)

3a. Nanoparticle biotransformation and degradation

- PL3**
8:00-8:35 Concepts of safety assessment of engineered nanomaterials (ENM)
Günter Oberdörster (*University of Rochester, U.S.A.*)
- O3a-1**
8:45-9:00 How do oxide nanomaterial dispersions evolve in an in vitro assessment?
Omar Lozano, J. Mejia, J-P. Piret, D. Jacques, C. Saout, J-M. Dogné, O. Toussaint, S. Lucas (*Research Centre for the Physics of Matter and Radiation, Belgium*)
- O3a-2**
9:00-9:15 Interaction of fibrinogen and albumin with titanium dioxide nanoparticles of different crystalline phases
Arianna Marucco, I. Fenoglio, F. Turci, B. Fubini (*University of Torino Dip, Italy*)
- O3a-3**
9:15-9:30 An effective photothermal therapy against cancer cells and deep tissue imaging with targeted NIR QD
Ravindran Girija Aswathy, B. Sivakumar, Y. Nagaoka, Y. Yoshida, T. Maekawa, D. Sakthi Kumar (*Bio Nano Electronics Research Center, Japan*)
- O3a-4**
9:30-9:45 Safety of nanovectors: Cytotoxicity assessment of New self-emulsifying multiple w/o/w nanoemulsions
Estelle Sigward, N. Mignet, P. Rat, M. Dutot, D. Scherman, D. Brossard, S. Crauste-Manciet (*INSERM, France*)
- O3a-5**
9:45-10:00 Surface ligand dependent fate and toxicity of ZnO np in HEPG2 cells
Dorota Bartczak, M-O. Baradez, H. Goenaga-Infante, D. Marshall (*LGC, UK*)
- 10:00-10:30 Coffee-break

Session 12: Regulation and standardization in nano-products

(Chair: Maila Puolamaa)

12a. Regulation

- PL12a**
10:30-11:05 Regulatory aspects of nanomaterials in REACH
Maila Puolamaa (*DG Enterprise and Industry, European Commission, Brussel*)
- O12a-1**
11:15-11:30 Legal issues of the environmental safety regulation in the sphere of nanotechnology in Russian federation
Ekaterina Belokrylova (*Udmurt State University, Russia*)

- O12a-2**
11:30-11:45 Regulation and safety implementation of nanotechnology for chemical enterprises in the Central Europe space
Andreas Falk, S. Hartl, F. Sinner (*BioNanoNet Forschungsgesellschaft mbH, Austria*)
- O12a-3**
11:45-12:00 Defining occupational and consumer exposure limits for nanomaterials - first experiences from REACH registrations
Karin Aschberger, F. M. Christensen (*European commission-JRC, Italy*)
- O12a-4**
12:00-12:15 Development of an integrative program of Nanosafety: Promote the Coordination Between Industries and Risk Assessor
Claude Emond, S. Kouassi, F. Schuster (*BioSimulation Consulting Inc, U.S.A, University of Montreal, Canada*)
- 12:30-13:30** Lunch

Session 8: Life cycle analysis, recycling, waste management and disposal

(Chair: Bernd Nowack)

- PL8**
13:30-14:05 Life cycle considerations for assessing environmental risks of nanomaterials
Bernd Nowack (EMPA, Switzerland)
- O8a-1**
14:15-14:45 Incineration of nanowastes: The implications from a life cycle perspective
Tobias Walser, L.K. Limbach, R. Brogioli, E. Erismann, L. Flamigni, B. Hattendorf, M. Juchli, F. Krumeich, C. Ludwig, K. Prikopsky, M. Rossier, D. Saner, A. Sigg, S. Hellweg, D. Günther, W. J. Stark (*Institute of Environmental Engineering, Switzerland*)
- O8a-2**
14:45-15:00 Preliminary evaluation of risks related to waste incineration of polymer nanocomposites
Lex Roes, M. K. Patel, E. Worrell, C. Ludwig (*Utrecht University, The Netherlands*)
- O8a-3**
15:00-15:15 Safe and environmentally-friendly management and final elimination of wastes harbouring nano-objects, or likely to release nanoparticles
Thierry Meunier, D. Vendittl & S. Durecu (*Groupe S  ch   Environnement, France*)
- O8a-9**
15:15-15:30 Investigation of the life cycle of nanoparticles by means of [^{44,45}Ti]TiO₂ and [^{110m}Ag]Ag⁰ – Research Project nanoTrack
Heike Hildebrand, K. Franke, A. Freyer, E. Bilz, R. Mehnert, E. Mai, C. Isaacson, K. Schirmer, A. Ammann, L. Sigg (*Helmholtz-Zentrum Dresden, Germany*)
- 15:30-16:00** Coffee-break

Session 10: Commercial equipment*(Chair: Robert Muir)*

- O10a-1**
16:00-16:15
Optical instrumentations for Nanosafety
Nathalie Vollmer (*HORIBA Scientific, France*)
- O10a-2**
16:15-16:30
NanoScan SMPS - a Novel, Portable Nanoparticle Sizing and counting Instrument
Torsten Tritscher, T.J. Krinke, A. F. Zerrath, E. Filimundi, O. F. Bischof (*TSI GmbH, GTSI Incorporated, Germany, U.S.A*)
- O10a-3**
16:30-16:45
Detecting & characterizing nanomaterials in complex matrices & airborne
Byron J. Cheatham (*CytoViva, USA*)
- O10a-4**
16:45-17:00
High Resolution Portable Scanning Mobility Particle Sizer Designed for best practice nano exposure measurements
Brian Steer, B. Gorbunov, R. Muir (*Naneum Ltd., UK*)
- O10a-5**
17:00-17:15
Nanoparticle Tracking Analysis (NTA): A tool in toxicology and environmental fate assessment of nanomaterial
Phil Vincent, P. Hole, P. Peotta, S. Capracotta, B. Carr (*NanoSight Ltd.,UK*)
- O10a-6**
17:15-17:30
Magellan: innovative detection, traceability and characterization of Nanoparticles traces in liquid media
Stéphane Aït Oumeghar, P. Nagtegale, D. Jacob (*Cordouan Technologies, France*)
- 20:00-23:00
Cocktail Party at La Bastille

ROOM B**Session 2: Detection and identification of nanoparticles***(Co-chair: Olivier Le Bihan)***2c. Detection and measurement of nanoparticles in water**

- O2c-1** Measuring particle size distributions of nanoparticles in aqueous media using FFF and LIBD
8:45-9:00
Nataliya Fedotova, K. Ralf, B. Sinnet, G. Detlef (*ETH Zurich, Switzerland*)
- O2c-2** Lab-on a chip μ TAS (Micro Total Analysis Systems) for the High-throughput Measurement of Nanomaterial Solubility
9:00-9:15
Ratna Tantra (*National Physical Laboratory, United Kingdom*)
- O2c-3** Species selective pre-concentration and quantification of Ag, Au and Pd nanoparticles using cloud point extraction and graphite furnace atomic absorption spectrometry
9:15-9:30
Georg Hartmann, M. Schuster (*Technische Universität München, Germany*)
- O2c-4** Quantitative Analysis of Engineered Nanoparticles in Food and Environment
9:30-9:45
Frank Von der Kammer, S. Wagner, S. Legros, B. Meisterjahn, E. H. Larsen, K. Loeschner, J. Navratilova, T. Hofmann (*Univ. of Vienna, Austria*)
- O2c-5** Detection of nanoparticle heavy metal Pollutants in water by laser-induced breakdown spectroscopy (LIBS)
9:45-10:00
Cheikh-Benoit Faye, C. Dutouquet, T. Amodeo, E. Frejafon, P. Delalain, O. Aguerre-Chariol, N. Gilon-Delepine (*INERIS, France*)
- 10:00-10:30 Coffee-break

Session 2: Detection and identification of nanoparticles*(Co-chair: Simon Clavaguera)***2d. Detection and measurement of nanoparticles in air**

- O2d-1** NanoBadge, a Tool for Engineered Nanoparticles Exposure Assessment.
10:30-10:45
Simon Clavaguera, M. Amdaoud, S. Jacquinet, S. Motellier, A. Guiot, L. Golanski, P. Capron (*CEA, France*)
- O2d-2** Measurement methods for the EC definition of nanomaterials
10:45-11:00
Luigi Calzolari, D. Gilliland, and F. Rossi (*European Commission - DG Joint Research Centre, Italy*)
- O2d-3**
11:00-11:15
- O2d-4** Detection of Nanoparticle agglomerates trapped in a low pressure RF (Radio-Frequency) plasma discharge using LIBS (Laser-induced Breakdown spectroscopy)
11:15-11:30
Christophe Dutouquet, G. Wattieaux, L. Meyer, E. Frejafon and L. Boufendi (*INERIS France*)

O2d-5 Characterization of manufactured TiO₂ nanoparticles
 11:30-11:45 Charles Motzkus, J. Idrac, T. Macé, S. Vaslin-Reimann, P. Ausset et M. Maillé
 (LNE, France)

Session 3: Toxicology

(Co-chair: Sophie Lanone)

3a. Nanoparticle biotransformation and degradation

O3a-6 ECSIN's methodological approach for hazard evaluation of engineered
 11:45-12:00 nanomaterials
Lisa Bregoli, F. Benetti, E. Sabbioni, (ECSIN, ITALY)

O3a-7 Degraded nanocomposites: a combined physico-chemical and toxicogenomics
 12:00-12:15 approach in caco-2 cells
Odette Prat, M. Fisichella, F. Berenguer, G. Steinmetz, M. Auffan, J. Rose
 (CEA, France)

O3a-8 Pristine graphene activates macrophages to produce cytokines/chemokines via
 12:15-12:30 TLR- and NF-kappa B-related signalling pathways
Taotao Wei, H. Zhou, K. Zhao and C. Chen (Chinese Academy of Sciences,
 China)

12:30-13:30 Lunch

Session 3: Toxicology

(Co-chair: Claude Emond)

3b. Nano bio interactions modeling

O3b-1 Assessment of different methods studying the impact of carbon nanomaterials
 13:30-13:45 on platelet function
Julie Laloy, F. Mullier, S. Robert, L. Alpan, J. Mejia, J-P.Piret, N. Bailly, S. Lucas,
 B. Chatelain, O.Toussaint, B. Masereel, S. Rolin & J-M. Dogné (University of
 Namur, Belgium)

O3b-2 Iron oxide nanoparticles show no toxicity in terms of comete assay in
 13:45-14:00 lymphocytes: a promising vehicle for nitric oxide releasing nanocarrier in
 =>(OP3a-54) biomedical applications
 R. de Lima, J. Luiz Oliveira, P. Sayuri Kaneko Murakami, M. A. M. Molina, R. Itri,
 P. Haddad, Amedea B. Seabra (Universidade de Sorocaba, Brazil)

O3b-3 Translocation of SiO₂ nanoparticles across human bronchial epithelial cells
 14:00-14:15 Isabelle George, S. Vranic, S. Boland, F. Marano, A. Baeza-Squiban (Université
 Paris Diderot, France)

O3b-4 Development of a dose-controlled multiculture cell exposure chamber for
 14:15-14:30 efficient delivery of airborne and engineered nanoparticles.
Akrivi Asimakopoulou, Emmanouil Daskalos, N. Lewinski, M. Riediker, E.
 Papaioannou, A. G. Konstandopoulos (Aerosol and Particle Technology
 Laboratory, CPERI/CERTH, Greece)

Panel discussion: Toxicology*(Moderator: Claude Emond)*

- 14:30-15:30 **Toxicology: what is the time-scale to identify hazard classes for the engineered nanoparticles? Application to nanomaterials safe by design**
 Claude Emond (*University of Montreal, Canada*), Günter Oberdörster (*University of Rochester, USA*), Gunnar Johanson (*Karolinska Institutet, Sweden*) Daniel Bernard (*ARKEMA, France*)
- 15:30-16:00 Coffee-break

Session 3: Toxicology*(Co-chair: Peter Hoet)***3b. Nano bio interactions modeling**

- O3b-5**
 16:00-16:15 In vitro, ex vivo and in vivo translocation of titanium dioxide nanoparticles through the gastrointestinal barrier, toxicological consequences
Emilie Brun, Nathalie Herlin-Boime, G. Veronesi, B. Fayard, A-M. Flank, M. Carrière (*CEA-CNRS, France*)
- O3b-6**
 16:15-16:30 Variation in silver nanoparticles toxicity in the presence of phenolic compounds
Alina Martirosyan, A. Bazes, Y-J. Schneider (*Institute of Life Sciences, Belgium*)
- O3b-7**
 16:30-16:45 Development of a PBPK model for ionic and nanoparticulate silver
Gerald Bachler, N. von Goetz, K. Hungerbühler (*ETH Zurich, Switzerland*)
- O3b-8**
 16:45-17:00 Predictive tests to evaluate oxidative potential of engineered nanomaterials.
Mara Ghiazza, E. Carella, S. Oliaro Bosso, M. Tomatis, I. Corazzari, M. Cristina Paganini, S. Livraghi, F. Viola, A. Marucco, B. Fubini, I. Fenoglio (*University of Torino, Italy*)
- O3b-9**
 17:00-17:15 Comparison of Toxicity of Uncoated and Coated Silver Nanoparticles
Kathy C Nguyen, P. Rippstein, J. Tan, A. F. Tayabali (*Health Canada, Canada*)
- O3b-10**
 17:15 -17:30 Nano-titanium dioxide modulates the dermal sensitization potency of DNCB
Stijn Smulders, V. De Vooght, S. Hussain, S. Boland, B. Nemery, P. HM. Hoet, J. AJ Vanoirbeek (*KU Leuven, Belgium*)
- O3b-11**
 17:30-17:45 Using a PBPK model to study the influence of different characteristics of nanoparticles on their biodistribution
Dingsheng Li, C. Emond, G. Johanson, O. Jolliet (*School of Public Health, University of Michigan, U.S.A*)
- O3b-12**
 17:45-18:00 Cytotoxicity of solid lipid nanoparticle and nanostructured lipid carriers with local anaesthetic dibucaine for topical application.
Nelson Durán, R. de Melo Barbosa, C. Moraes G. da Silva, T. dos Santos Bella, D. Ribeiro de Araújo, P. D. Marcato, E. de Paula (*State University of Campinas, Brazil*)
- 20:00- 23:00 Cocktail Party at La Bastille

ROOM C

Session 6/7: Secure industrial production and protection technology*(Co-chair: Jesús López de Ipiña Peña)***6a. Secure industrial production**

- O6a-6**
8:45-9:00 In situ synthesis of anti-bacterial nanocomposite coatings by nanosafe-by-design sputtering process
Alain Billard, E. Monsifrot, I. Sayah, F. Sanchette, F. Schuster (*DEPHIS SARL, France*)
- O6a-7**
9:00-9:15 Secure Process through fonctionnalisation: elaboration of organic composites reinforced with aligned carbon nanotubes grown on carbon fibers
Stéphanie Patel, Y. Magga, M. Pinault, D. Porterat, G. Deniau, C. Reynaud, M. Mayne-L'Hermite (*CEA, France*)
- O6a-8**
9:15-9:30 Criteria and guiding principles for the precautionary design and for improved recyclability of engineered nanomaterials
Michael Steinfeldt (*University of Bremen, Germany*)
- O6a-9**
9:30-9:45 From Safe Nanomanufacturing to Nanosafe-by-Design processes
Frédéric Schuster, F. Lomello (*CEA, France*)
- 10:00-10:30 Coffee-break

Session 11: Risk management for OHS experts*(Chair: Paul Schulte)**(Co-chair: Olivier Witchger and Eric Drais)*

- PL11**
10:30-11:05 Risk management for OHS experts
Paul A. Schulte (*National Institute for Occupational Safety and Health, USA*)
- O11a-1**
11:15-11:30 Horizon-scanning and Identification of emerging risks among nanotech-companies
Steffen Foss Hansen, H. V. Kristensen, A. Baun (*Technical University of Denmark, Denmark*)
- O11a-2**
11:30-11:45 Risk assessment of nanomaterials and nanoproducts – adaptation of traditional approaches
Jutta Jahnel, T. Fleischer, S. B. Seitz (*Karlsruhe Institute of Technology, Germany*)
- O11a-3**
11:45-12:00 OHB based risk assessment method for powders and nanomaterials
Malcom Staves, Laurent Gridelet, P. Delbecq, L. Hervé, G. Fayet, D. Fleury, S. Kowal (*SOPROREAL, France*)
- O11a-4**
French approach for characterizing potential emissions and exposure to

12:00-12:15 aerosols released from nanomaterials in workplace operations
Catherine Durand, O. Witschger, O. Le Bihan, E. Zimmermann, A. Marchetto, M. Reynier, D. Charpentier (*CEA, France*)

O11a-5 Concerns related to Safety Management of Engineered Nanomaterials
 12:15-12:30 Amela Grosa, T. Meyer (*Ecole Polytechnique Fédérale de Lausanne, Switzerland*)

12:30-13:30 Lunch

Session 11: Risk management for OHS Experts

(Co- chair: Eric Drais)

O11a-6 Design of a prevention approach: from representations to action, the case of
 13:30-13:45 nanomaterials
Catherine L'Allain, S. Caroly, E. Drais (*Laboratoire PACTE, Université de Grenoble, INPG, France*)

O11a-7 From nanomaterials risk perception to risk management: last literature survey
 13:45-14:00 lessons
Eric Drais (*INRS, France*)

O11a-8 French registry of workers handling engineered nanomaterials as an Instrument
 14:00-14:15 of integrated system for surveillance and research
Irina Guseva Canu, O. Boutou-Kempf, L. Delabre, S. Ducamp, Y. Iwatsubo, J-L. Marchand, and E. Imbernon (*French Institute for Public Health Surveillance, France*)

Session 12: Regulation and standardization in nano-products

(Co-chair: Olivier Salvi)

12 b. Standardization

PL12b NanoSTAIR project: Establishing a process and a platform to support
 14:30-14:45 standardization for nanotechnologies
Olivier Salvi, E. Fréjafon (*EU-VRi, Germany*)

O12b-1 Current International Standardization in particle characterization and release
 14:45-15:00 testing
Michael Stintz, L. Hillemann (*Institute of Process Engineering and Environmental Technology, Germany*)

O12b-2 Overview of standardization activities in Europe (CEN) and in relation with ISO
 15:00-15:15 and OECD
Jean-Marc Aublant (*LNE, France*)

O12b-3 Managing Operation Procedures in collaborative projects
 15:15-15:30 Marie-Gabrielle Ollivier Beuzelin, A. Hool (*Ecole Polytechnique Fédérale de Lausanne, Switzerland*)

Wednesday 14 November 2012

ROOM C

15:30-16:00 **Coffee-break**

Session 8: Life cycle analysis*(Co- chair: Mickael Riediker)*

- O8a-5**
16:00-16:15 LCA Modelling of Engineered Nanomaterials: a framework for establishing sound inventory data of production & releases of nano objects along the life cycle.
Roland Hischier (*EMPA, Switzerland*)
- O8a-6**
16:15-16:30 LCA case studies of nanotechnology-based applications in the project NanoSustain
Michael Steinfeldt (*University of Bremen, Germany*)
- O8a-7**
16:30-16:45 Life cycle assessment of a self-cleaning coating based on nano TiO₂-polyurea resin applied on aluminum panel
Martina Pini, A. M. Ferrari, R. Gamberini, P. Neri, B. Rimini (*EN & TECH - Italy*)
- O8a-8**
16:45-17:00 Impact assessment of nano activated polymeric membranes
Stefano Zuin, P. Scanferla, W. Wennekes, J. E. Wong, K. De Sitter, C. Dotremont, I. Genne (*Venice Research Consortium, Italy*)
- O8a-4**
17:00-17:15 Environmental exposure modelling of Engineered Nanomaterials and comparison to their corresponding bulk/total material flows
Tianyin Sun, F. Gottschalk, K. Hungerbühler, B. Nowack (*EMPA, Switzerland*)

Session 6/7: Secure industrial production*(Co- chair: Frédéric Schuster)***6b. Protection technology**

- O6b-1**
17:15-17:30 Evaluating performance of containment equipment designed for handling manufactured nanomaterials by use of nanoparticle marker
Sébastien Artous, P. Bombardier, S. Derrough, D. Locatelli, P. Nobile, C. Durand (*CEA, France*)
- O6b-2**
17:30-17:45 Experimental and numerical comparative study of the containment of airborne nanoparticles and gas released inside a safety cabinet
Vincent Cesard, E. Belut, C. Prévost (*INRS, France*)
- O6b-3**
17:45-18:00 Experimental evaluation of the resistance of protective gloves against titanium dioxide nanoparticles in solution under conditions simulating occupational use
Ludwig Vinches, P. Dolez, K. J. Wilkinson, S. Hallé (*Université de Montréal, Canada*)
- 20:00-23:00 Cocktail Party at La Bastille

Thursday 15 November

MINATEC AUDITORIUM

Session 4: Environmental interactions

(Chair: Jérôme Rose)

4a. Transport, transformations and trophic transfer

- PL4** Environmental Interactions
8:00-8:35 **Jérôme Rose**, M. Auffan, P. Chaurand, J. Labille, D. Borschneck, A. Masion, H. Miche, C. Botta, C. Geantet, E. Puzenat, P. Afanasiev, E. Lecelrc, J. Garric, F. Manuela, B. Vollat, P. Noury, K. Abbaci, J-Y Bottero (*CEREGE, France*)
- O4a-1** Effect of TiO₂ nanoparticles on larval development of swallowtail within a food chain
8:45-9:00 **Miyoko Kubo-Irie**, M. Yokoyama, K. Takeda, M. Irie (*Tokyo University of Sciences, Japan*)
- O4a-2** Role of water temperature in the fate, transport, bioavailability of engineered nanoparticles in aquatic environments
9:00-9:15 **Seyed Mohammad Majedi**, H. K. Lee, B. C. Kelly (*National University of Singapore, Singapore*)
- O4a-3** MWCNT accumulation during hydroponic exposure of wheat and rapeseed: quantification and distribution
9:15-9:30 **Camille Larue**, M. Pinault, B. Czarny, D. Georgin, E. Flahaut, N. Bendiab, M. Mayne-L'Hermite, V. Dive, F. Taran, M. Carrière (*CEA-CNRS, France*)
- O4a-4** Benthic Food Chain studies with TiO₂ nanoparticles.
9:30-9:45 **Carl W. Isaacson**, L. Sigg', A. Amman, and K. Schirmer (*EAWAG, Switzerland*)
- O4a-5** Modeling the fate of nano-TiO₂ in the Rhone river – the importance of hetero aggregation with natural colloids
9:45-10:00 **Antonia Praetorius**, J. Labille, M. Scheringer, J-Y. Bottero, K. Hungerbühler (*ETH Zürich, Switzerland*)
- 10:00-10:30 Coffee-break

Session 5: Nanomaterial release

(Chair: Tinh Nguyen)

5a. Release by environmental stress

- PL5** Quantitative Studies of Photo-induced Surface Accumulation and Release of Nanoparticles in Polymer Nanocomposites
10:30-11:05 **Tinh Nguyen**, D. Stanley, S. Rabb, D. Banerjee, X. Gu, L. L. Yu, L. Sung, and J. W. Chin (*NIST, USA*)
- O5a-1** Scenarios and methods that induce protruding or released CNT's after degradation of composite materials
11:15-11:30

Wendel Wohlleben, L. Cena, S. Hirth, G. Cox, Ž. Tomović, T. Peters (*BASF SE, Germany*)

O5a-2 Monitoring migration and transformation of nanomaterials in polymeric composites during climatic aging
11:30-11:45
Gemma Vilar, E. Fernández-Rosas, G. Janer, E. Mas del Molino, M. Busquets-Fité, V. Puntès, S. Vázquez-Campos (*LEITAT Technological Center, SPAIN*)

O5a-3 Release of nanoparticles from textiles during washing
11:45-12:00
Bernd Nowack, Lena Windler, C. Lorenz, N. von Goetz, K. Hungerbühler, M. Heuberger (*EMPA, Switzerland*)

O5a-4 Release of TiO₂ particles from paints containing pigment TiO₂ and/or nano-TiO₂ by weathering experiments
12:00-12:15
Ahmed Al-Kattan, A. Wichser, R. Vonbank, S. Brunner, A. Ulrich and B. Nowack (*EMPA, Switzerland*)

O5a-5 Release of CeO₂ nanoparticles upon aging of acrylic wood coating
12:15-12:30
Lorette Scifo, P. Chaurand, A. Masion, M. Auffan, M-A. Diot, J. Labille, J-Y. Bottero and J. Rose (*Tecnalia, France*)

12:30-13:30 Lunch

Session 4: Environmental interactions

(Co-Chair: Jean-Yves Bottero)

4b. Biodegradation/ biointeractions

O4b-1 Aged TiO₂-based nanomaterial used in sunscreens: implications on *ESCHERICHIA COLI* sensitization to toxic metal
13:30-13:45
Catherine Santaella, B. Allainmat, F. Simonet, J. Labille, C. Geantet, J. Rose, W. Achouak (*CNRS-CEA, France*)

O4b-2 Characterizing nanoparticles reactivity: structure-Photocatalytic activity relationship
13:45-14:00
Jordi Piella, N. Bastus, V. Puntès (*ICN, Spain*)

O4b-3 TiO₂ nanoparticles in cosmetic sunscreen: effect of aging on physico-chemical and cytotoxic properties of both plain formulation and extracted nanoparticles
14:00-14:15
Manon Rossano, N. Hucher C. Picard, M. Grisel, F. Le Foll (*URCOM, CNRS, France*)

O4b-4 Assessment of Environmental Exposure to Nanomaterials through mesocosms experiments
14:15-14:30
Mélanie Auffan, M. Tella, L. Brousset, J. Issartel, C. Pailles, B. Espinasse, E. Artells, A. Thiery, C. Santaella, W. Achouack, A. Masion, J. Rose, J-Y. Bottero (*CEREGE-CNRS, France*)

- O4b-5** 14:30-14:45 Biotransformation of Carbon Nanotubes and Fullerenes by Horseradish Peroxidase.
Deborah Xanat Flores-Cervantes, J. Hollender, Hans-Peter E. Kohler (*EAWAG, Switzerland*)
- O4b-6** 14:45-15:00 Cobalt, titanium dioxide and nanosilver nanoparticles cause skeletal damages in sea urchins at pluteus stage
Antonietta Morena Gatti, C. Gambardella, S. Ferrando, L. Gallus, P. Ramoino, C. Falugi (*University of Genoa, Italy*)
- O4b-7** 15:00-15:15 Comparison of effects on crustaceans: carbon nanoparticles and molybdenum nanowires
Anda Baumerte, G. Sakale, J. Zavickis, M. Knite, L. Putna, M. Balode, A. Mrzel (*University of Latvia, Slovenia*)
- 15:30-16:00 Coffee-break

Session 4: Environmental interactions

(Co-chair: Laurent Charlet)

4c. Biologicals effects of NMs

- O4c-1** 16:00-16:15 Concerns over aluminum oxide nanoparticle based applications: an ecologically inspired study using environmentally relevant isolates and medium
Sunandan Pakrashi, N. Chandrasekaran, A. Mukherjee (*VIT University, India*)
- O4c-2** 16:15-16:30 The role of silver and vanadium to the ecotoxicity of silver vanadate nanowires decorated with silver nanoparticles
Gisela de Araújo Umbuzeiro, M. Coletty Artal, F. Kummrow, R. Dias Holtz, O. Luiz Alves (*State University of Campinas, Brazil*)
- O4c-3** 16:30-16:45

Session 3: Toxicology

(Co-chair: Marie Carrière)

3d. Tissues and environnement

- O3d-5** 17:00-17:15 Violacein/poly- ϵ -caprolactone/chitosan nanoparticles against bovine mastitis: antibacterial and ecotoxicity evaluation
Gisela A. Umbuzeiro, E. Berni, G. Nakazato, F. I. Vacchi, N. Durán (*UNICAMP, Brazil*)

- O3d-6**
17:15-17:30 Evaluation of multi walled carbon nanotubes eco(geno)toxicity using the amphibian larvae of *xenopus laevis*
Laury Gauthier, F. Mouchet, C. Gancet, A. Perrault, F. Bourdiol , E. Flahaut, P. Puech, E. Pinelli, J-C. Boutonnet (CNRS, NAUTILE, France)
- O3d-7**
17:30-17:45 Molecular interference between titanium from NP-TiO₂ nanoparticles and iron homeostasis in E. coli
Caroline Fauquant, Isabelle Michaud-Soret, A-N. Petit, N. Herlin-Boime, P.-H. Jouneau, S.Ollagnier de Choudens (CEA-CNRS, France)
- O3d-8**
17:45-18:00 Fullerene nanoparticles C₆₀ and C₆₀(OH)₁₈₋₂₂: Assessment of biological activity using bacterial cells and rat liver mitochondria as model systems
Sandra M. Santos, R. A. Videira, L. Ferreira, A. M. Dinis, F. Peixoto, A. S. Jurado (University of Coimbra, Portugal)
- 18:15 Conclusion

ROOM B

Session 3: Toxicology*(Co-chair: Naohide Shinohara)***3c. Nanoparticles translocation and barrier**

- O3c-1**
8:00-8:15 Tissue Distribution and clearance of Titanium Dioxide nanoparticles after intravenous administration and Intratracheal instillation
Naohide Shinohara, H. Fukui, N. Danno, T. Ichinose, K. Honda, M. Gamo (*AIST, Japan*)
- O3c-2**
8:15-8:30 A dynamic co-culture model resembling the alveolo-capillary barrier to study toxicity and translocation of nanoparticles
Katrien Luyts, B. Nemery and P. H.M. Hoet (*K.U.Leuven, Belgium*)
- O3c-3**
8:30-8:45 Communicating Nanotoxicology: three Evaluations using *in vitro* central nerve models
Fumihide Kanaya, S. Hanada, Y. Inoue, Y. Manome, K. Fujioka, (*National Center for Global Health and Medicine, Japan*)
- O3c-4**
8:45-9:00 Comparative study of neurologic effects of nano-TiO₂ versus SiO₂ after direct intracerebral exposure in mice
Aur lie Balvay, N. Thieriet, L. Lakhdar, A. Bencsik (*ANSES, France*)
- O3c-5**
9:00-9:15 Interactions of functionalized MWCNTs with primary neural cells from different brain regions: site-specific effects
Cyrill Bussy, J. Boczkowski, S. Lanone, M. Prato, A. Bianco, K. Kostarelos (*Centre for Drug Delivery Research, UK*)
- O3c-6**
9:15-9:30 Specific uptakes and damages induced by polystyrene nanobeads according to surface chemistry
Vincent Paget, S. Dekali, T. Kortulewski, R. Grall, S. Chevillard, A. Braun P. Rat, G. Lacroix (*INERIS, France*)
- O3c-7**
9:30-9:45 Application of *in vitro* BBB model to measure permeability of nanoparticles
Sanshiro Hanada, K. Fujioka, Y. Inoue, F. Kanaya, Y. Manome, K. Yamamoto (*National Center for Global Health and Medicine, Japan*)
- O3c-8**
9:45-10:00 3D model of air-blood barrier for the study of nanoparticles translocation
Samir Dekali, V. Paget, C. Gamez, P. Rat and G. Lacroix (*INERIS/ C-TAC, France*)
- 10:00-10:30 Coffee-break

Session 3: Toxicology*(Chair: Shuji Tsuruoka)***3c. Nanoparticles translocation and barrier**

- O3c-9**
11:15-11:30 Assessment of cytotoxicity, intracellular uptake and intestinal absorption of amorphous silica nanoparticles in the Caco-2 in vitro human intestinal barrier model
Agnieszka Kinsner-Ovaskainen, C. Ubaldi, I. Cydzik, F. Simonelli, E. Alloa, M. Ceridono, D. Gilliland, N. Gibson, J. Ponti, F. Rossi (*JRC, Italy*)
- O3c-10**
11:30-11:45 Influence of the length of imogolite-like nanotubes on their cytotoxicity and genotoxicity towards human dermal cells
Wei Liu, P. Chaurand, C. Di Giorgio, M. De Méo, A. Thill, M. Auffan, A. Masion, D. Borschneck, F. Chaspoul, P. Gallice, A. Botta, J-Y. Bottero, J. Rose (*CEREGE-CNRS, France*)
- O3c-11**
11:45-12:00 Effect of Different surface charge based superparamagnetic iron oxide nanoparticles (SPION) on Biodistribution in Rat and *Ex vivo* Protein fishing
Usawadee Sakulkhu, L. Maurizi, A. Gramoun, M-G. Beuzelin, J-P Vallée, G. Coullerez, H. Hofmann (*École Polytechnique Fédérale de Lausanne, Switzerland*)
- O3c-12**
12:00-12:15 An Impedance-based High-throughput Method for Evaluating the Cytotoxicity of Nanoparticles
Mihaela Roxana Cimpan, T. Mordal, J. Schölermann, U. Pliquet, E. Cimpan (*IKO, Faculty of Medicine and Dentistry, Norway*)
- O3c-13**
12:15-12:30 The use of differentiated human respiratory epithelial cells in inhalation toxicology of nanomaterials
Frieke Kuper, A. Reus, M. Gröllers, F. van Acker, I. Kooter (*TNO Quality and Safety, The Netherlands*)
- 12:30-13:30 Lunch

Panel discussion*(Moderator: Alexei Grinbaum)*

- 13:30-14:30 **The opinion of the Civil Society: international NGO and european labor unions regarding nanomaterials approaches**
Alexei Grinbaum (*CEA, France*), Aida Ponce Del Castillo (*ETU, Brussel*), Luisa Filipponi (*NANOPINION, Brussel*), Alain Lombard (*VivAgora, France*)

Session 3: Toxicology*(Co-chair: Frédéric Bois)***3d. Tissues and environment**

- O3d-1**
14:30-14:45 ROS evaluation for a series of CNTs and their derivatives using ESR method with DMPO
Shuji Tsuruoka, K.Takeuchi, K. Koyama, M. Endo, H. Matsumoto, N. Saito, Y. Usui, D. W. Porter, V. Castranova (*Shinshu University, Japan*)
- O3d-2**
14:45-15:00 Toxicity towards lung cells and Escherichia Coli: Impact of nanoparticle dispersion status
Nathalie Herlin-Boime, Marie Carrière, S. Pigeot-Rémy, A. Casanova, C. Guillard, J-C. Lazzaroni, D. Atlan (*CEA-UJF, France*)
- O3d-3**
15:00-15:15 Noteworthy Interaction of TiO₂ Nanoparticles (Anatase) with Bacterial Cells under Dark Conditions
Swayamprava Dalai, N. Chandrasekaran, A. Mukherjee (*VIT University, India*)
- O3d-4**
15:15-15:30 Determination of endotoxin concentration by different test methods: influence of sample preparation and particle interference on test reliability
Stijn. Smulders, J.-P. Kaiser, P. Wick, P. Hoet (*K.U.Leuven, Belgium*)
- 15:30-16:30 Coffee-break
- 18:15 End of the conference

ROOM C**Session 9: Ethics and societal issues***(Chair: Alexei Grinbaum)*

- PL9**
10:30-11:05 Nanotechnology and the narratives of responsibility
Alexei Grinbaum (*CEA, France*)
- O9a-1**
11:15-11:30 Nanotechnology, responsibility and responsible innovation
Christopher Groves (*ESRC, Cardiff University, United Kingdom*)
- O9a-2**
11:30-11:45 The social context of nanotechnology and Regulating its uncertainty: a nanotechnologist approach
Vincent Jamier, I. Gispert, V. Puentes (*Centre for NanoBioSafety and Sustainability, Spain*)
- O9a-3**
11:45-12:00 The Nano@School project: a new pedagogical initiative to increase the awareness of nanosciences and nanotechnologies in the classrooms
Francine Papillon, E. Excoffon, A. Bsiesy, J. Chevrier (*CEA Grenoble, France*)
- O9a-4**
12:00-12:15 Latest research results on the effects of nanomaterials on humans and the environment: DaNa - Knowledge Base Nanomaterials
Clarissa Marquardt, K. Nau, H.F. Krug, D. Kühnel, B. Mathes, V. Richter, S. Scholz, C. Steinbach (*KIT, Germany*)
- 12:30-13:30 Lunch

Session 5: Nanomaterial release**5b. Release by mechanical stress***(Co-chair: Francois Tardif)*

- O5b-1**
13:30-13:45 Release-ability of nano fillers from different nanomaterials (Toward the acceptability of nanoprodukt)
Luana Golanski, A. Guiot, S. Motellier, A. T. Saber, F. Tardif, P. Capron (*CEA-Liten, France*)
- O5b-2**
13:45-14:00 Nanomaterial release from nanocomposites during reworking process
Virginia Gómez, M. Levin, S. Irusta, M. Dal Maso, J-M. Santamaría, K. A. Jensen, I. K. Koponen (*INA, Spain*)
- O5b-3**
14:00-14:15 Nanoparticle release from Nanocomposites due to mechanical treatment at two stages of the life-cycle
Daniel Göhler, A. Nogowski, P. Fiala, M. Stintz (*Institute of Process Engineering and environmental Technology, Germany*)

- O5b-4**
14:15-14:30 On character of coarse, fine and ultrafine particles in automotive brake wear debris
Jana Kukutschová, P. W. Lee, V. Matějka, K. Malachová, E. Veselá, Pavlína Peikertová, K. Čabanová, M. Vaculík, P. Filip (*Nanotechnology Centre at VŠB, Czech Republic*)
- O5b-5**
14:30-14:45 Abrasion tests on MWCNT composites: influence of CNT dispersion state and filler/matrix interface
Maxime Pras, J. Duchet-Rumeau, J-F. Gerard, L. Golanski, A. Guiot (*UMR CNRS, France*)
- O5b-6**
14:45-15:00 Generation of aerosols during the mechanical solicitation of materials: development of an experimental set-up and applications
Christophe Bressot, N. Shandilya, O. Aguerre-Chariol, M. Morgeneyer, O. L. C. Le Bihan (*INERIS, France*)
- O5b-7**
15:00-15:15 On nanoparticles release from polymer nanocomposites for applications in lightweight automotive components
James Njuguna, S. Sachse, F. Silva, S. Michalowski and K. Pielichowski (*Centre for Automotive Technology, Cranfield University, UK*)
- O5b-8**
15:15-15:30 Abrasion behavior of an epoxy-based nanocomposite with raw- and functionalized carbon nanotubes
Lukas Schlagenhauf, Bryan T.T. Chu, J. Buha, F. Nüesch, J. Wang (*Swiss Federal Institute for Materials Testing and Research, Switzerland*)
- 15:30- 16:00 Coffee-break

Session 5: Nanomaterial release

5c. General and other release mechanisms

(Co-chair: Wendel Wohlleben)

- O5c-1**
16:00-16:15 Nanomaterial dustiness - a comparison between three methods
Keld Alstrup Jensen, M. Levin, I. K. Koponen, D. Bard, A. Kelley, G. Burdett, S. Bau, O. Witschger (*National Research Centre for the Working Environment, Denmark*)
- O5c-2**
16:15-16:30 NanoRelease – Developing Methods to Measure Release of Nanomaterials from Solid Consumer Products
Lie Chen, M. Hill, R. Canady (*Health Canada, Canada*)
- O5c-3**
16:30-16:45
- O5c-4**
16:45-17:00 Characterization and quantification of nanoparticle release from commercial available spray products containing engineered nanoparticles
Sabrina Losert, M. Lattuada, K. Hungerbühler, A. Ulric (*EMPA, Switzerland*)

- O5c-5** Behavior of nanoparticles during high temperature treatment (incineration type)
17:00-17:15 Samir Derrough, G. Raffin, D. Locatelli, P. Nobile, C. Durand (*CEA, France*)
- O5c-6** Fate of CNT-epoxy composite during incineration
17:15-17:30 Ulrika Backman, J. Lyyräinen, T. Kettunen, J. Leskinen, O. Sippula, A. Auvinen, J. Jokiniemi (*VTT Technical Research Centre of Finland, Finland*)
- O5c-7** Release of TiO₂ nanoparticles from cement during their life cycle: step of use
17:30-17:45 Nathan Bossa, J. Rose, P. Chaurand, O. Aguerre-Chariol (*INERIS, iCEINT, France*).
- 18:00 Conclusion
- 18:15 End of the conference

Conference opening: PL0a

INNOVATION PERSPECTIVES THROUGH NANOMATERIALS: THE INTEGRATED APPROACH AT CEA

F. Schuster¹, CEA Saclay, 91191 Gif -sur-Yvette, France

Industrial needs in terms of multifunctional components are increasing. Several sectors are concerned, ranging from mature high volume markets like automotive applications, high added value parts like space & aeronautic components or even emerging activities like new technologies for energy. Also are concerned domains with a planetary impact like environment and new products and functions for health and safety of people. Nanotechnologies could play a key role in promoting innovation in design and realisation of multifunctional products for the future, either by improving usual products or creating new functions and new products. Nevertheless, this huge evolution of the industry of materials could only happen if the main technological and economic challenges are solved with reference to the societal acceptance. Those concern the mastering, over the whole life cycle of the products, of the potential risks, by an integration of the elaboration channels, while taking into account recycling. Nanomaterials are present in most of the programs of the CEA. True to its culture of risk management, CEA has developed over the past ten years an integrated, multidisciplinary management of the potential risks associated with nanomaterials. Initially, this approach was strongly supported by the European Commission. The need to develop technological infrastructures

Conference opening: PL0b

CODES, STANDARDS AND REGULATIONS IN PREPARATION AT WORLD-WIDE LEVEL

Dr Françoise D. ROURE, *Chair, Committee "Technologies and society"*, High Council for Economy, Industry, Energy and Technologies, Ministère de l'économie et des finances, Ministère du redressement productif
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Questions about open source and open access to databases dealing with nanomaterials become crucial in order to guarantee accountable development for territories. These developments focus on nano technologies and their applications, like synthetic biology. Some countries started with a huge investment in metrology and behavior characterization for such nanoscale materials, natural, synthetic, hybrid and functionalized. Inclusion of such bio-sourced materials at nanoscale in the standard definition and future prospects about a boosted dynamic of standardization at European and International level will impact straightly organization and the knowledge provision, in the field of biotechnologies. In particular, those implemented by efficient and evolutionary regulatory framework will address industrial nanotechnologies applications while preserving competitiveness requirements in the global industrial world.

Nanomaterial description and the related knowledge management becomes a major scientific, economic and societal stake. Presentation will refer to the work in progress in this field.

Conference opening: PL0c

REGULATION, RISK, AND THE GLOBAL NANOTECHNOLOGY WORKPLACE

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Novel properties of engineered nanomaterials (ENMs) present opportunities for important innovations in energy, medicine, and other applications. Current research on the nanotechnology industry indicates its downstream expansion at a rapid pace, while toxicological research and best practices for environmental health and safety are still being developed. Current regulatory approaches rely largely on industry self-regulation and self-reporting. In this context of soft regulation, ENM companies play an important role in safeguarding human health and the environment. This presentation assesses company-reported environmental health and safety (EH&S) practices and reports industry views on nanomaterial risks and regulation. Analysis is based on responses from 78 company participants in a 2009-2010 international survey of private companies that use and/or produce ENMs.

This research found relatively high levels of uncertainty and perceived risk, and these perceptions were not accompanied by expected evidence of risk avoidant practices or preferences for regulatory oversight. A majority of companies reported “lack of information” as a significant impediment to implementing nano-specific safety practices but were not found to consistently incorporate widely available guidance, defaulting instead to an “industry knows best” position.

While companies reported practices that in aggregate span all elements of a government-recommended hierarchical approach to exposure controls, nano-specific practices within and outside this approach were reported less frequently than general chemical hygiene practices. Product stewardship and waste management practices – product end-of-life practices – were reported less frequently than most other environmental health and safety practices. Additionally, in the absence of safe-handling regulations, companies reported nano-specific health and safety programs that were narrow in scope. The study found little evidence for expected significant variance in practices and risk perceptions across countries.

This research suggests that government guidance is not reaching industry. While industry reluctance toward regulation might be expected, their own reported unsafe practices and recognition of possible risks suggest a more top-down approach from regulators is needed to protect workers and the environment. They also suggest that more attention to environmental health and safety is necessary, especially with regard to product end-of-life practices.

Conference opening: PL0d

ARE SPECIFIC REGULATIONS FOR NANOMATERIALS EFFICIENT?

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With the increase of the production and the uses of the “*so called nanomaterials*”, more and more specific regulations take place at national, regional or international level to protect customers and citizen environment from the uncertainties regarding their risk assessment and safety evaluation. There is a priority need to identify by clear unequivocal descriptions what can be considered as nanomaterials for regulators and manufacturers.

It should be stressed that nanomaterials is a material categorization by the size of its internal or external structure. It does not imply a specific risk, nor does it necessarily mean that this material has new hazard properties compared to its constituents.

There is evidence that there is a change in some properties of the material at the nanoscale size which is, for instance, due to the increased surface-to-volume ratio. The external nano-scale size of some nanomaterials constituent parts could influence their bio-distribution and distribution kinetics in an organism, and cause harm to humans and the environment. These particular nanomaterials are named nano-objects (nano-plates, nano-fibers or nano-particles,) with one, two, or three external sizes at the nanoscale (see ISO TC229 / TS 80004-1, TS 80004-2, TS 80004-4).

When considering any definition for nanomaterials, size is the predominant feature and is the most suitable measurand. In most of the working definitions proposed for regulatory purposes, the size refers to one or more external dimensions within a specified size range. A defined size range would facilitate a uniform interpretation. However, if an upper limit of 100 nm, or approximately 100 nm, is commonly used. There is no scientific evidence to qualify the appropriateness of this value.

So any nanomaterial should be described by its size distribution, including the protocol used for the measurement. This requires that adequate validated methodologies are available for carrying out measurements at the nanoscale (i.e. below 1 micron? Including sampling, sample preparation and dispersion) to ensure comparability of results.

But why regulatory definition should be limited to purposely-designed nanomaterials (e.g. engineered or manufactured nanomaterials), excluding incidental nanomaterials?...

Conference opening: PL0e

INNOVATION GOVERNANCE: CHALLENGES IN THE FIELD OF NANOTECHNOLOGIES

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The public debate about nanotechnologies as one of the key innovation drivers for smart materials has changed a lot over the past decade. Coming from a discussion about futuristic visions for health, energy and mobility, the well-known marketed products today are more often found in the field of easy-to-clean surfaces, paints and textiles, sunscreens and construction materials. The expectations of policy makers, researchers and engineers for a prosperous future and the development of innovative technologies in Europe are still high, but the public debate is getting more and more silent.

In consequence, consumer perceptions are decreasing, the knowledge is fading out, and uncertainty occurs. New data about public perception suggests that there is still a positive basic attitude towards Nanotechnologies in Europe, but the debate is getting increasingly diverse, complex and ambiguous.

It seems that the innovation in research and technical development has to be accompanied by societal innovation how to deal with emerging technologies.

Significant steps have been made by Risk Governance Frameworks and voluntary Codes of Conduct embedding sustainability aspects such as environmental protection, occupational health and consumer's safety in very early stages of the development process. Broad stakeholder groups have been involved successfully in several dialogue projects. However, the proceedings did not reach the broader public and communication patterns of the applying industry did not change visibly. The focus on potential risks could be one of the reasons why governance approaches did not last sustainable and why an open communication strategy seems to be so difficult. However, the public debate will turn negative if silence is the dominant pattern of communication. The talk will provide some suggestions how to think in concepts of Innovation Governance and how to develop dialogue strategies for researchers, industry and policy makers.

N° PL1

**ENGINEERED NANOMATERIALS: FROM SOURCE TO DOSE AND THE ROLE OF MEASUREMENT DEVICES
AND MEASUREMENT STRATEGIES**

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Technical processes and their products may release material into the environmental compartments water, soil and air, which may cause unwanted effects. In general, this process chain consists of various parts and can be described in terms of emission of a material from a source, transmission within or through compartments, and immission into a microenvironment at a receptor. The latter can be considered as exposure, which can result in uptake and a biological effect.

Focusing on human exposure to engineered nanoparticles (ENPs) via air, we can distinguish process of emission/release, transport and immission, that determine what part of the generated ENP will result in actual concentration at the receptor, i.e. the breathing zone of worker or consumer.

For initial risk assessment, the likelihood of exposure can be estimated by the potential for emission or release. Many tiered approaches for exposure assessment include phases where process characteristics are evaluated with respect to emission or release potential, e.g. VCI, 2011. Currently, only a few processes to mimic release have been standardized, e.g. dustiness, abrasion, thermo-gravimetric analysis. Therefore, the use of real-time devices that measure number-, surface area- or mass concentration to assess emission or release has been incorporated in the tiered approach measurement strategies. Since these type of devices do not characterize the particles, interpretation of the results in view of ENP emission or release is not straightforward.

For a comprehensive health-relevant exposure assessment, there is consensus that a multi-metric approach is needed; Total and size resolved surface area concentration and particle number concentration, mass concentration in 'nano' and inhalable respirable ranges, particle shape and agglomerate structure and composition, and (bio)activity. So far, these required features are not met by a single device, so a suite of devices is needed. Currently, a range of devices are being developed that can assess breathing zone concentrations more accurately (www.nano-device.eu).

Exposure models may provide the appropriate concentrations in the breathing zone, however, much more information is needed for a dose estimate. Firstly, the duration of the exposure, or the concentration-time profile is an important factor for the translation of breathing zone concentration into dose estimates. Secondly, actual respiration and consecutive deposition both in terms of efficiency and location of the respiratory tract are -in addition to aerosol characteristics- very much affected by breathing patterns and the anatomy of the airways. Breathing patterns, will be determined by the physical stress related to the work or task, whereas the anatomy is an individual factor. Currently, some new proposals for deposition conventions, including ultrafine/ nano particles have been adopted [4], which enable a more accurate calculation of the deposited dose.

Consequently, so called 'contextual information' linked with any data on (breathing zone) concentrations is a key factor for interpretation of these data. Information about the task, e.g. type, physical stress, duration, etc. is essential with respect to dose estimates, thus this type of information should be collected as part of a measurement strategy.

O1a-1

OBSERVATION OF NANOAEROSOL RELEASE FROM ELECTRONIC HOUSEHOLD PRODUCTS

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Safety issues of nanomaterials are of concern in workplaces and consumer use. Many nanoproducts are commercialized and some of them are household products in daily use. In-vitro and in-vivo toxicities of nanomaterials have been widely conducted. Recently, some exposure assessments in workplace were reported. However, studies on consumer exposure to household products are not well reported.

In this paper, we focused on the release of nanoaerosols from electronic household products such as hand dryer, hair iron, and air purifier. To quantify the amount of nanoaerosol release, a test chamber system was designed. It consisted of a cube-shape chamber, a pure air supply unit, real-time aerosol monitoring instruments, and a filter sampler. The volume of the chamber was 93 L and it was enough to install a full-scale test product. The total particle number concentration was monitored by using a condensation particle counter (CPC, TSI model 3010) and the wide-range particle size number distribution was measured by using a wide-range particle spectrometer (WPS, MSP model 1000XP). In high-release cases, a filter sampler was used to analyze particle morphology and elemental composition.

Hand dryer and hair irons were selected as test products. The hand dryer had a filter coated with silver nanoparticles. Two kind of hair iron were tested for comparison. One had a heat plate coated with silver nanoparticles and the other had a heat plate without silver nanoparticles. The hair iron can heat its ceramic plate by 190°C in 1 min. These electronic test products were operated under test.

The test was conducted as follows. First, a test product was set in the test chamber. Prior to main test, the chamber was purged with a pure air of 10 L/min to minimize a background particle concentration. Initial particle concentration in the chamber was higher than 3,000 particles/cm³ and the background level of <1 particles/cm³ was achieved after 90-min purging.

When the test hand dryer was turn on, the particle number concentration abruptly increased up to a peak value and then exponentially decreased down to the background level. The peak particle concentration ranged 10-20 particles/cm³. Repetition of operation of the test electronic hand dryer showed a periodic particle release, implying that the test hand dryer generates a small amount of nanoaerosols during the use period, particularly in the turn on stage.

When the test hair iron coated with silver nanoparticles was turn on, the particle number concentration dramatically jumped at ~40,000 particles/cm³, which was much higher than the indoor particle concentration at the test environment. Thereafter, particle concentration shortly dropped about 1/3-1/2 of peak value and slowly increased up to a peak level. Then, it slowly decreased. A similar release trend was also observed for the test hair iron without silver nanoparticles. The nanoaerosol release seemed to be caused by heat plate.

N°O1a-2

AEROSOL EMISSION ASSESMENT DURING SOLDERING PROCESS

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Assessing the risk of nanomaterial exposure harmful effects has been of a great concern during the last few years. Some literature has been focused on manufacture and final product incorporation steps during nanomaterial cycle-life. However, nanomaterial occupational exposure is also possible during ordinary everyday processes. Therefore, detection, identification and assessment of these source processes, especially in workplaces, are needed.

Soldering, the join of two electronic components by using a filler tin-based metal alloy, is a widely spread conventional process in electronica industry. A small amount of flux based in colophony Rosin is present in soldering wires and low temperature lead-based joining alloys are still common in some occupational environments. Even though the continuous exposure to colophony fumes was studied and it is known that has harmful effects as bronchial asthma[1] and dermatitis the generation of nanoparticles from this process is still not clear.

In the present work the release of nanoparticle-laden aerosols with variable load of metals in soldering fumes has been studied. For that two kinds of solder alloys, with and without lead in their composition, have been chosen in order to assess particle release during wire soldering process. Total number particle concentration during soldering has been measured by using CPC and OPC and aerosol size distribution has been measured with SMPS. Further characterization of the collected particles has been done by transmission and scanning electron microscopy and energy dispersive X-ray spectroscopy.

Aerosol size distributions show primary particles with diameters below 100 nm, on the other hand aggregates with diameters below 1 µm consisting on metallic nanoparticles in a flux matrix were found in collected samples. EDX analysis shows lead and tin signals as could be seen in Fig. 1.

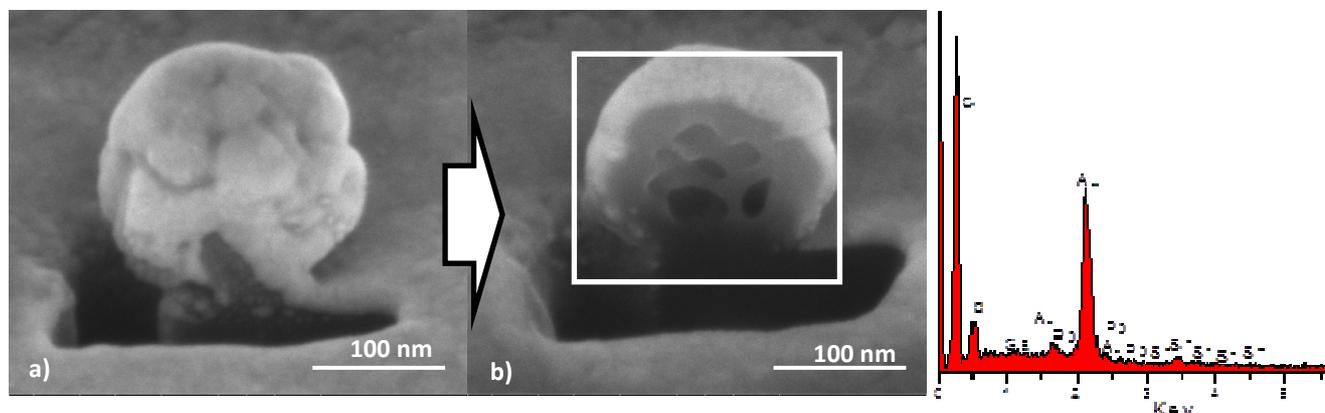


Fig. 1. SEM images of particles collected during soldering using a SnPbCu alloy a) before and b) after ion milling and EDX spectra of the highlighted area.

Acknowledgments: This work was supported by Spanish MICINN through research projects MAT2008-01319/NAN and PSE-2008-420000-3. Financial support of the FP7 European project NanoValid (#263147) is also acknowledged. V.G. acknowledges the FPU scholarship AP2008-02067.

1. McDonald, J.C., et al., *Incidence by occupation and industry of acute work related respiratory diseases in the UK, 1992-2001. Occupational and Environmental Medicine*, 2005. 62(12): p. 836-842.

N°O1a-3

EXPOSURE TO AEROSOLS ASSOCIATED WITH CLEANOUT OPERATIONS OF A REACTOR PRODUCING NANOCOMPOSITE THIN FILMS EMBEDDED WITH SILVER NANOPARTICLES

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Despite the growing number of studies on the risks associated with nanomaterials, there are still very limited workplace exposure data if the entire life cycle of nanomaterials is considered. Among the many exposure scenarios identified in industry or research laboratories, those on maintenance and cleaning of equipments and workstations should not be underestimated.

This study investigates the potential for exposure via inhalation from cleanout operations by sandpapering of a reactor producing nanocomposite thin films embedded with silver nanoparticles (~ 8 nm). Only few metallic pieces could be taken apart from the reactor, for which the sandpapering took place inside a constant velocity hood located in a different room. The operators carried personal protective equipment (gloves, facial mask respirator) as the reactor's room was not equipped with special built ventilation compartment or local exhaust ventilation.

According to Witschger *et al.* (2012), the measurement strategy was based on: (1) real-time measurement of the size distribution, number and lung-deposited surface-area concentrations; (2) integrated sampling for mass size distribution and respirable concentrations at different fixed locations distant from the source; (3) breathing zone respirable sampling on the operators; (4) short time sampling at the source for electron microscopy (EM) observation and identification. Gravimetric and chemical analyses for select metals (Ag, Fe, Ni, Cr, Cu, Co) were performed for all filters. The background issue has been addressed through the near-field and far-field approaches. The sandpapering operation generates at the source an elevated concentration of airborne silver containing particles, which are transferred to the laboratory atmosphere. Close to the source, the silver mass concentration was 89µg/m³, while the respirable concentration measured at 1 m and on the operators were 550 and 3000 times lower respectively. The Ag mass size distribution was bimodal, with maxima at ~300 nm and ~ 4 µm (Fig. 1), while the number size distribution was in the sub-micrometer range. EM images and EDS analysis confirm the results.

As very few data are available to assess occupational exposures to nano-silver it is thought that this work would be of particular interest for health risk assessment.

In addition, the study results call for the need to consider potential exposures during cleanout operations of the nanomaterials synthesis reactors by establishing real data, as the investigated environment is thought to be reflective of many academic research laboratories.

Witschger O., Le Bihan O., Reynier, M., Durand C., Marchetto A., Zimmermann E., Charpentier D. (2012) Recommendations for characterizing potential emissions and exposure to aerosols released from nanomaterials in workplace operations. Hygiène et sécurité du travail, 226:41-55 (in French).

N°O1a-4

CHARACTERIZATION OF NANO-AEROSOLS DURING THERMAL IMAGING OF NANOPOLYMER COATED PLATES IN A WORKPLACE

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Airborne nano-particles (NP) emitted in a workplace undergo continuous transformations due to physical processes. As a result aerosol characteristics change, leading to spatial and temporal variation of the number concentration and size distribution across the workplace. It is important to understand these transformation processes, because it influences workers exposure to NP.

The major aim of this study was to identify if unwanted ablation occurs during the thermal imaging process resulting in the release of NP in the occupational environment. Minor objectives were to 1) find out the main source for NP emissions (the imager itself or the exhaust ventilation unit); 2) determine emission characteristics (mass, number, size) for different digital plates and different writing settings; 3) examine the influence of R.H. on NP measurements; 4) check if a different exposure pattern (full versus line exposure) influences NP emission.

The experiments were performed in an industrial R&D plant where thermal imaging was performed with a commercial imager on commercial plates. The imager was positioned in a polyethylene tent (24 m³) with no room ventilation to decrease the influence of other particle emitting processes in the laboratory and creating a smaller workplace ('worst-case scenario'). Simultaneous measurements were conducted both indoor and outdoor using dustmonitors with NanoChecks in order to determine background concentration. During thermal imaging variations were made in writing settings (drum speed and laser intensities) resulting in normal, underexposed or overexposed conditions. During each run several P-traks and Dusttraks were used to scan the imager to detect if emission sources were present. The sample inlets of the SMPS and APS were positioned in the vicinity of the operator and near the filtered exhaust ventilation unit.

Compared to the background concentration of 1.600 particles per cm³ increased concentrations of NP were measured during the imaging of different plates. Maximum concentrations reached 158.000 particles per cm³ and mean diameters for different plates varied between 64 and 88 nm (Figure 1).

In order to determine differences in emission characteristics (mass, number, size) between the different plates, variations were made in the pattern (line or full exposure) and the writing settings (. The results showed significant differences between plates for NP and PM4 emissions at conditions of full or line exposure. During full exposure more NPs are emitted, which tend to coagulate and grow faster than during line exposure when fewer particles are present. Comparing mass and number concentration data from different writing settings it was found that for positive plates overexposure resulted in higher concentrations than at normal exposure, in contrast with negative plates. By increasing the laser intensity on a negative plate more particles in the coating are stabilized while with positive plates this will result in a more instable coating. Particle number concentration decreased when the sample flow was being dried using a nafion dryer. The influence of R.H.(%) on the particle measurements was checked using normal and overexposed conditions. The results showed that mass measurements were more influenced by R.H.(%) than number concentration measurements. Also the influence of the tent as a worst case setting for exposure assessment on concentration and size measurements was checked against a 'normal' setting (laboratory). Both mass and number concentrations were significant lower without the tent while no significant difference in size distribution was found. Without the tent the particles could disperse faster leading to lower concentrations.

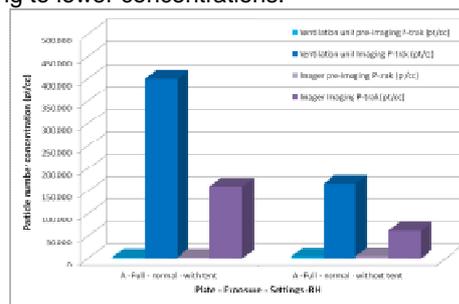
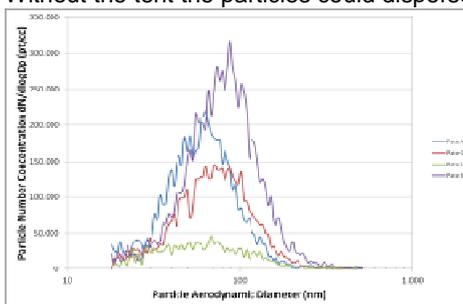


Figure 1: Size distributions for different plates Figure 2: Number concentrations at full exposure with and without the tent

More results will be discussed in the paper

N°O1b-1

A MODULAR TOOL FOR ANALYZING CASCADE IMPACTORS DATA TO IMPROVE EXPOSURE ASSESSMENT TO AIRBORNE NANOMATERIALS

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Cascade impactors are widely used to provide particle size distributions for the study of aerosols in workplaces and ambient air [1]. Several devices can be employed (e.g. Marple, Andersen, Sioutas, Berner, Moudi, DLPI, etc.), with variable flow rates, cutoff diameters and number of channels.

In the frame of exposure assessment to airborne particles, one of their main advantages is the possibility to perform further off-line analysis (e.g. electron microscopy, physical-chemical characterization by XRD, ICP-MS, etc.) on the collected samples according to particle size. However, the large channel width makes the particle size distributions not enough size-resolved. Furthermore, in spite of the sharpness of the collection efficiency curves, the existence of an overlap between stages renders data interpretation difficult [1], especially in the case of nanoparticles where calculation of lung-deposited particle concentrations is advised by ISO [2].

This work aim was to develop a modular programme allowing the inversion of data stemming from cascade impactors based on the mass (or any quantity) collected on each impaction stage. Through a precise description of the collection efficiency curves of the different stages, the software provides a continuous curve (from 100 to 1000 points) using the Markowski method, and more particularly the Twomey iterative algorithm, according to several publications about inverse problems in cascade impactors. An additional option consists in determining the experimental error at each point of the inverse curve, performed by realizing several consecutive inversions.

The inversion procedure was first tested and optimized for the case of the Sioutas personal sampler. Validation of the calculation was performed considering theoretical aerosols. Figure 1a presents input and output distributions the case of a bimodal aerosol, Figure 1b is an example of output with confidence interval.

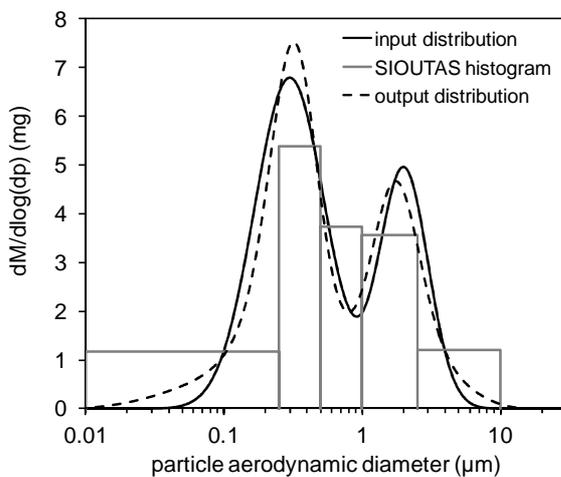


Figure 1a

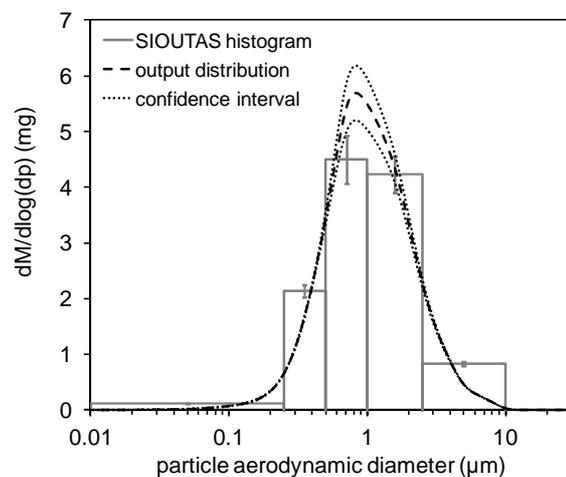


Figure 1b

[1] ISO/TR 12885 (2008). *Nanotechnologies — Health and safety practices in occupational settings relevant to nanotechnologies.*

[2] (EN) ISO 13138 (2012). *Air quality — Sampling conventions for airborne particle deposition in the human respiratory system.*

N° O1b-2

**EXPLORATORY STUDY ON TWO STATISTICAL METHODS TO ANALYSE TIME RESOLVED DATA
OBTAINED DURING NANOMATERIAL EXPOSURE MEASUREMENTS**

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Most strategies of measurements that are suggested at the international level to assess workplace exposure to nanomaterials rely on devices measuring, in real time, airborne particles concentrations (according different metrics). Since none of the instruments to measure aerosols implemented can distinguish a particle of interest to the background aerosol, the statistical analysis of time resolved data requires special attention. So far, several approaches have been used for statistical analysis in the literature. This ranges from simple qualitative analysis of graphs to the implementation of more complex statistical models like the Autoregressive Integrated Moving Average (ARIMA) time series models. To date, there is still no consensus on a particular approach and the current period is always looking for an appropriate and robust method. In this context, this exploratory study investigates two different statistical methods to analyse time resolved data: one is based on time series analysis following the ARIMA approach, and the other one is based on a Bayesian probabilistic approach. To investigate and illustrate the use of the two statistical methods particle number concentration data from a workplace study that investigated the potential for exposure via inhalation from cleanout operations by sandpapering of a reactor producing nanocomposite thin films have been used. In this workplace study, the background issue has been addressed through the near-field and far-field approaches and several size integrated and time resolved devices have been used. The analysis of the results presented here focuses only on data from the condensation particle counters used: TSI's Condensation Particle Counter 3007 located far-field and P-Trak located at the source of the released particles.

The ARIMA approach is to create a statistical model per data series and then compare these two models (source and background) between them. This allows determining the quantity of airborne particles released during the observed task (sandpapering) while taking into account the background aerosol. The Bayesian probabilistic approach allows a probabilistic modelling of data series, and the observed task is modelled in the form of probability distributions. The probability distributions issuing from time resolved data obtained at the source can be compared with the probability distributions issuing from the time resolved data obtained far-field, leading in a quantitative estimation of the airborne particles released at the source when the task is performed. The obtained results highlight strengths and weaknesses of each statistical method depending on the objectives of the study. Moreover, good practices for the gathering of real time measurements are elaborated for industrial hygienists who might want to use statistical analysis for data exploitation.

Beyond obtained results, this exploratory study indicates that the analysis of the results requires specific experience in statistics.

N°O1b-4

APPROACH TO THE EXPOSURE ASSESSMENT OF MWCNT BY CONSIDERING SIZE DISTRIBUTION AND OXIDATION TEMPERATURE OF ELEMENTAL CARBON

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Multi-walled carbon nanotubes (MWCNTs) having many beneficial characteristics are categorized as nanomaterial, but health risks from exposure to MWCNTs like other nanomaterials are also concerned. Therefore, quantitative procedure for exposure assessment of MWCNT is required.

As an approach to the exposure assessment of MWCNTs, we characterized and determine MWCNTs using an aerosol carbon monitor. Other carbonaceous particles, such as SWCNT, diesel exhaust particles (DEP) and ambient particulate matter (APM) were also characterized by the same method to distinguish MWCNTs from other carbonaceous particles. Carbonaceous particles were oxidized at three steps of oven temperature in the carbon analysis without optical correction. Each oxidized fraction was assigned EC1, EC2 and EC3 as different types of elemental carbon (EC). Aerosol samples were collected on five stages by a Sioutas cascade impactor (SCI), which collects size-segregated airborne particles having aerodynamic diameters >2.5 µm, <2.5 µm, <1.0 µm, <0.5 µm, and < 0.25 µm.

The amounts of ECs determined by the monitor coincided with the mass of MWCNTs calibrated by a microbalance. The portion of oxidized carbon at each temperature depended on the sample characteristic such as diameter of MWCNT. As MWCNTs aggregate/agglomerate easily, they were observed as micron size particles. On the other hand, EC contained in APM is mainly observed in fine particles. Therefore, the size of aerosol was a good parameter to distinguish the MWCNTs from other particles, especially APM.

Exposure assessment of MWCNT can be conducted as follows (Figure 1): 1) Analyze ECs at three different temperature, target MWCNT material should be analyzed in advance to confirm the oxidation pattern of the material; 2) Collect size-segregated particles, the fractions respirable micron-size and smaller than 500 nm particles are necessary; 3) Confirm the presence of MWCNT by EC pattern and by SEM; 4) Determine MWCNT by the amount of EC2 and/or EC3.

This procedure can be applied to the downstream users applying materials, such as composite or fibers, containing MWCNTs.

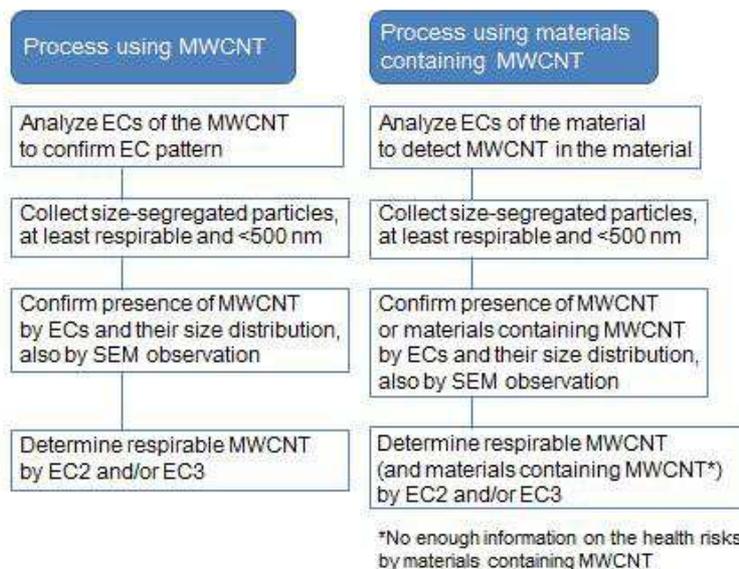


Figure 1 Probable approach for quantitative exposure assessment of processes handling MWCNTs.

N° O1b-5

IS EXHALED BREATH CONDENSATE REPRESENTATIVE OF DEEP LUNG AND SUITABLE FOR EXPOSURE ASSESSMENT TO NANOPARTICLES?

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The use of engineered nanoparticles (NP) is expanding very fast worldwide, but there is still a lack of clear answer on their potential toxicity. Moreover, there is special concern for the inhalation route of exposure based on ultrafine particles health effects. Special concern arises also from the asbestos drama. In this context, it is of main importance to be able to evaluate NP exposure of individuals, especially at work places where exposure might be potentially elevated. Assessment of external exposure must be addressed but this approach should be completed by biomonitoring which is the only way to take into account the use of protective devices and individual variability. In the case of insoluble NP, toxicokinetics data tend to prove that very few NP would be found in blood or urine following inhalation. This is why other biological media have to be explored.

Exhaled breath condensate (EBC) is sampled in a non-invasive way, and contains a small part of non-volatiles compounds that emanate from the respiratory tract. Our hypothesis is that NP might be found in EBC following inhalation and might be used as specific biomarkers of exposure to engineered NP, even though standardization issues of EBC still need further investigations.

One major question regarding EBC is whether this biological medium is representative of the alveolar region of the lung which is of main concern for nanoparticle potential toxicity but can not be examined non-invasively. One of the goals of this study is therefore to compare broncho-alveolar lavages (BAL) and exhaled breath condensates from the same subjects in terms of nanoparticles composition.

This will be investigated in the starting project EXPO-NANO supported by the Canceropole Lyon Auvergne Rhone Alpes (CLARA) in collaboration between St Etienne hospital and Jean Monet university (LINA), and the LABM in CEA Grenoble. BAL and EBC will be collected from 100 patients who have a medical prescription for LBA in the pneumology department of St Etienne hospital over one year. The BAL will be analyzed in the LINA and the EBC in the LABM.

EBC will be collected with the RTube device from Respiratory Research. Nanoparticles determination in EBC will be performed thanks to elemental composition determination by inductively coupled mass spectrometry after acidic mineralization and granulometric analysis performed by dynamic light scattering. Additional characterization will be obtained on selected samples with scanning electron microscopy.

Results in EBC will be compared to those obtained in BAL after inter-laboratory comparison for analytical procedures.

This study should bring new information on the feasibility to measure nanoparticles in EBC and on EBC representativeness of deep lung. This is a first step in the development of potentially new biomarkers of exposure to nanoparticles. In the future, such an approach should be gone into detail with exploratory studies in subjects potentially exposed to engineered nanoparticles.

N°O1c-1

PRO ET CON ANALYSIS OF OCCUPATIONAL EXPOSURE ASSESSEMENT TOOLS AND CONCEPTS FOR NANOMATERIALS

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There is an urgent need for simple and yet robust scientific methods to evaluate the potential of occupational exposure related to the production and application of nanomaterials. A number of alternatives to traditional exposure assessment have recently been explored and proposed for nanomaterials. Examples of these include the "Control Banding Nanotool" developed to assess and control the risks of nanomaterials, the more holistic "Swiss precautionary matrix", and the first order quantitative risk assessment tool, NanoSafer. Here we review these and other tools and we discuss various elements of the tools (input data requirements, exposure evaluation and handling to reduce exposure) as well as specific pros and cons. Most of the tools provide a transparent and comprehensible approach to assess occupational exposure, but the majority of them are based on purely qualitative considerations about occupational settings. A few methods include specific advice on risk management going well beyond what is normally considered in traditional exposure assessment. A disadvantage in most of the existing concepts is that their data requirements are fairly high. In some cases the technical and scientific procedures to determine them is inconclusive or non-existing. Some of the concepts are furthermore based on purely theoretical considerations and too time-consuming to apply in reality. We provide a set of recommendations for what regulators and risk assessors need to consider before selecting and applying one or the other tool in a given situation and call for further application and development of these tools in the support regulatory decision-making. The aim should be to develop a tiered approach with a purely qualitative, a semi-quantitative, and purely quantitative tool, respectively which can be employed depending on available data and user background. See figure 1 for an illustration of how this could be envisioned in regard to the on-going development of the Danish NanoSafer tool.

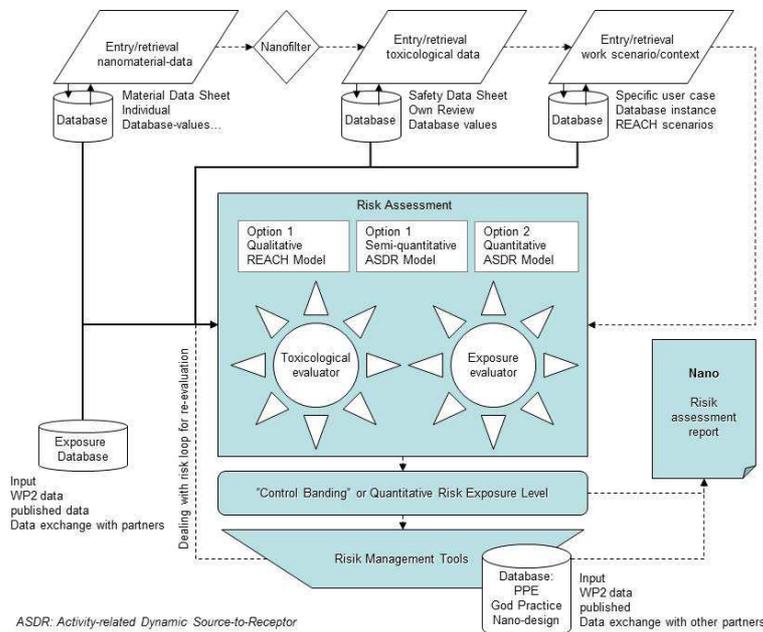


Figure 1 : Schematic Illustration of how one of the evaluated tools, i.e. NanoSafer could potentially incorporate various exposure assessment models e.g. a qualitative REACH model, a semi-quantitative ASDR model and a quantitative ASDR model termed option 1,2 and 3, respectively

N°O1c-2

URBAN AIR POLLUTION OF OSTRAVA REGION BY NANOPARTICLES

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Ostrava, as a capitol of Nord-Moravian and Silesian region (Czech Republic), belongs among the cities with very high pollution by airborne particles. Despite the legislation saying that concentration of PM10 should not exceed the limit 50 µg/m³ more than 35 days per year, in the year 2011 such situation occurred 126 times. Several health impacts of air pollution are reported.

The reason of pollution is complex and still under discussion. The principal contributions are heavy industries including metallurgy, machinery, energy production and chemical industry, together with the high density transport and extended local heating by solid fuels. The situation is worsened by orography and unfavourable dispersion conditions. The composition of sources implies the possibility of increased nanoparticles content in pollution.

Originally, only the sum of all solid particulate matter was measured. As a result of growing knowledge about behaviour and impact of airborne particulate matter, the attention is drawn to smaller particles. PM10 measurement is complemented by PM2,5 and recent investigation show that probably PM1 will replace it. While the smaller particles can more easily enter the human body, the future concern of air pollution is nanoparticles. This is why the pilot study of the nanoparticles concentration in the air of Ostrava region was performed.

Measurement was conducted at two different localities in the city of Ostrava. The first (Mariánské Hory), represents the place with high the contribution of transport, the second (Radvanice) represents the locality with extended local heating and proximity of metallurgical complex.

For the measurement of the particles concentration and size distribution of the Fast Mobility Particle Sizer Spectrometer (measuring range from 5.6 nm to 560 nm) was used. For the sampling, the device Nano-ID Select with 12 channels in the size range 0.001 - 35 micron-, was used. Characterization of chemical composition of samples was performed by ICP-MS technique.

In different days, different values were measured in both concentration and fraction distribution of nanoparticles. Three maxima (~10 nm, ~40 nm, ~100 nm) were observed, but in different days, different maximum predominated and one-, two- and three-modal distributions were found. Air contamination by nanoparticles is not homogeneous and local extremes (maxima) lasting about minutes were observed frequently, what indicates that there are small local contamination sources in nearness of sampling point. In the proximity of industry, higher concentrations were measured in the majority of cases

N°O1c-3

NANOPRODUCT SIMPLIFIED DIRECT EXPOSURE ASSESSMENT TOOLKIT

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Industrials have to develop the safest nano products possible, considering numerous gaps of knowledge [^{1,2}] and experimental methodological frameworks [^{3,4,5,6,7,8}]. Expert consensus on uncertainty and complexity in nanomaterial safety [⁹] shows that the exposure side of the equation could be considered with fewer uncertainties. Consequently, we propose a simplified nano product exposure assessment toolkit, dedicated to highlight the exposure part of the equation. In fact, whatever on the toxicity of a nanoparticle, the exposure level is the first information, allowing to guide the industrials for safe nano product design and to decide consumers to accept or not this product.

We consider that nano product direct exposure (Ed) for consumers depends on:

1. The nanoparticle amount category (A)
2. The Emission potential (E), which depends on the product category (PC) and the use conditions
3. The Transfer potential (T), directly linked with the migration routes
4. The Deposit fraction (D), which depends on the exposure paths
The time cumulating/h (F) and the Context of the utilisation (C)

Every score is necessarily associated with one product and one scenario.

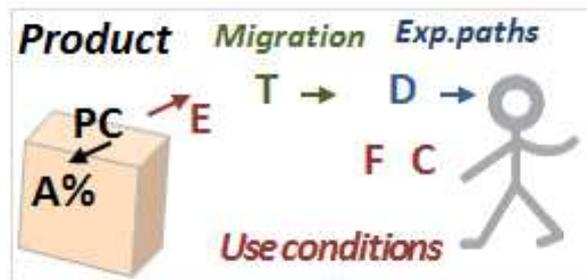


Fig.1. Nano product exposure parameters

A consensus approach: A set of nano products has been considered with “normal use” and “worst case” scenarios. A panel of six experts apply this framework to the case studies and produce observations for improving the exposure assessment toolkit. Then we will extend the number of case studies for improving robustness and sensibility of the framework. In a third step we will have to consider indirect exposure by food and water, via environmental paths.

First results: During the conference, the updated exposure framework will be discussed. All observations of the exposure experts from scientific community will be welcomed in order to contribute to deliver a pragmatic nano product exposure assessment toolkit.

¹ Nowack, *Is anything out there? Nanotoday*, 4, 11-12, 2009,

² FP7 nanEX

³ S.Y.Paik, *The Annals of Occupational Hygiene*, Vol 52, 6, p. 419-428

⁴ T.Eason & al. *Guidance to Facilitate Decisions for Sustainable Nanotechnology*, US EPA, Sept 2011

⁵ SF Hansen, *NanoRiskCat – A Conceptual Decision Support Tool for Nanomaterials*, danish EPA, Nov 2011

⁶ *Nanorisk Framework*, Environmental Defense – DuPont, Nano Partnership, June 2007

⁷ *Precautionary matrix for synthetic nanomaterials*, Office Fédéral Suisse, v 2.1, July 2011

⁸ Sorensen P.B. & al. *Conscious worst case definition for risk assessment, part I*, *Science of The Total Environment*; 408(18): 3852-9, 2010.

⁹ G. Hunt & al. *Building expert consensus on problems of uncertainty and complexity in nanomaterial safety*, *Nanotechnology Perceptions* 7, 82-98, July 2001

N°PL2

**DETECTION AND IDENTIFICATION: INSTRUMENTATION AND CALIBRATION FOR
AIR/LIQUID/SURFACE-BORNE ENGINEERED NANOPARTICLES**

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Sustainable nanotechnology requires environmental, health and safety studies of nanoparticles (Nano-EHS), involving physico-chemical characterization, exposure and toxicity, and abatement by filtration of nanoparticles. I will first describe my previous research on the development of the DMA-based submicron aerosol standard, and the certification of the NIST 60 nm and 100 nm nanoparticle size standards, leading to the two ISO standards for nanoparticle particle size distribution and number concentration. The development of the electrical aerosol analyzer has led to the elucidation of the nuclei mode and the accumulation mode of the atmospheric aerosols. Recently we have developed a Universal Nanoparticle Analyzer (UNPA) for measuring the primary particle size of the nanoparticle agglomerates in near real-time. By incorporating a filter sensor with the UNPA, the fractal dimension of the nanoparticle agglomerates can be determined. Since the air-borne nanoparticle instruments provide higher sensitivity and resolution than the liquid-borne instruments, various methods for dispersing liquid-borne nanoparticles for air-borne measurements will be described. Methods for depositing air-borne particles on surfaces will also be described, which provide a means for calibrating air-borne and surface-borne particle concentrations. The proper use of these instruments for exposure assessments at various workplaces will be presented.

David Y. H. Pui, a Distinguished McKnight University Professor, is the L.M. Fingerson/TSI Inc Chair in Mechanical Engineering and the Director of the Particle Technology Laboratory and of the Center for Filtration Research (CFR), University of Minnesota. CFR consists of 12 major filter manufacturers and end users supporting the filtration research. Dr. Pui has a broad range of research experience in aerosol science and technology and has over 220 journal papers and 20 patents. He has developed/co-developed 10 widely used commercial aerosol instruments. Dr. Pui is a fellow of the American Society of Mechanical Engineers (ASME) and a founding fellow of the American Association for Aerosol Research (AAAR), and is a recipient of the Smoluchowski Award (1992), the Max Planck Research Award (1993), the International Aerosol Fellow Award (1998), the Humboldt Research Award for Senior U.S. Scientists (2000), and the AAAR David Sinclair Award for Senior Scientists (2002). He received the Fuchs Memorial Award, the highest award for the aerosol discipline, at the International Aerosol Conference in Helsinki in 2010, conferred jointly by the American, German and Japanese aerosol associations. During 2006-2010, Dr. Pui served as the President of the International Aerosol Research Assembly (IARA) consisting of 16 international aerosol associations.

N°O2a-1

**TOWARDS THE EFFICIENT AND VERSATILE SYNTHESSES OF NANOMATERIALS FOR
NANOSAFETY INVESTIGATION**

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Nanoscience provides many opportunities to significantly improve materials properties and sustainability. The current and projected applications of engineered nanoparticles span a wide range of industrial and consumer sectors such as biomedicine, pharmaceuticals, cosmetics, new sources of energy, etc. However the environmental and human health risks of many nanomaterials remain unknown, and prioritizing how to efficiently assess their risks is essential.¹ The precautionary principle should be used by lowering the exposure (in terms of environment and human contact to the nanomaterials) to zero. In this approach, at the Nanomaterials Chemistry and Safety Laboratory (LCSN), the performance of personal protective equipment and filters exposed to nanomaterials is evaluated and tools for occupational exposure assessment to nanoparticles are under development.

The evaluation of the toxicity of the nanomaterials is rather complex because it is intrinsically connected to specific parameters of the nanomaterials² such as dissolution, chemical composition, size, shape, agglomeration state, crystal structure, specific surface area, surface energy, surface charge, surface morphology and surface coating. Therefore it is important to test the performance of personal protective equipment exposed to different type of nanomaterials. In this context, at LCSN a wide range of nanomaterials (core or core shell systems) was synthesized.

The different types of nanomaterials synthesized include i) metallic nanoparticles such as silver, nickel and gold, ii) oxide nanomaterials such as SiO₂, ZnO, and TiO₂, iii) semiconductor nanoparticles such as CdSe and InP, iv) organolanthanide based nanoparticles. Several procedures were used to synthesize the different nanomaterials such as wet chemical procedures (sol-gel, Stöber, precipitation), microwave and hydrothermal synthesis (autoclave). The synthesized nanomaterials have different features accordingly to the initial intended application, for example the core/shell systems ¹¹⁰Ag@SiO₂ and ⁵⁷Co@SiO₂ for radioactive labels, the SiO₂ nanoparticles doped with Alexa Fluor 680, the organolanthanide complexes encapsulated in SiO₂ nanoparticles for luminescent labels and the Fe₃O₄ nanoparticles for magnetic labels.

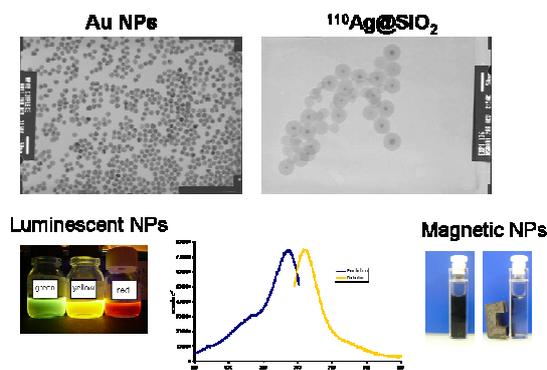


Figure 1. TEM images of Au (core) and of ¹¹⁰Ag@SiO₂ (core/shell) nanoparticles.

¹ "Risk assessment strategies as nanomaterials transition into commercial applications", M.S. Olson, P. L. Gurian, *J. Nanopart. Res.* (2012), 14, 786.

² "Toxicity of nanomaterials", S. Sharifi, S. Behzadi, S. Laurent, M. L. Forrest, P. Stroeve and M. Mahmoudi, *Chem. Soc. Rev.* (2012), 41, 2323–2343.

N°O2a-2

METAL IMPURITIES PROVIDE USEFUL TRACERS FOR IDENTIFYING EXPOSURES TO AIRBORNE SINGLE-WALLED CARBON NANOTUBE PARTICLES RELEASED FROM WORK-RELATED PROCESSES

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The purpose of this research is to devise practical methods for distinguishing process-related nanoparticle (NP) emissions from background. Electrostatic precipitation and filter-based approaches were investigated as methods of characterizing airborne manufactured nanomaterials during workplace exposure scenarios, by exploiting metal impurities which are present in SWCNT agglomerates at concentrations that are orders of magnitude higher than in ambient air particles. While direct readings of number, mass, and surface area concentrations are recommended as a first step in identifying workplace nanoparticle (NP) exposures, these methods do not provide information about morphology and chemical composition of the NPs being measured. Agglomerates of SWCNT material (Aldrich 698695) collected on PTFE filters using a cascade impactor were characterized using TEM and SEM. Image analysis determined that amorphous carbon comprises about 45% of the SWCNT agglomerates. Backscatter electron (BSE) analysis of dry-mounted SWCNT indicated the location of metal catalysts (Y, Ni) as bright spots associated with the amorphous carbon. Elevated concentrations of Y (2%) and Ni (13%) in the bulk sample were confirmed by ICP-MS determination. Bioaccessibilities of these catalysts and other metal impurities were determined using physiologically-based extractions at pH conditions relevant to both ingestion and inhalation pathways. Exposure measurements were performed by placing an aerosol-to-liquid particle extraction system (ALPXS; Meinhard) beside a Ro-Tap sieve shaker inside a self-ventilated hood, and positioning the air sampling intake about 25 cm from the source. As shaking intensity increased from the lowest to the highest setting (Figure 1), airborne Y increased from 27 ng/m³ to 1839 ng/m³ and airborne Ni increased from 151 ng/m³ to 8794 ng/m³. Background air concentrations of both elements were below detection limits (≤ 2 ng/m³). These observations indicate that catalysts and other metal impurities in manufactured NPs can be used as airborne tracers to identify emissions at source, provided that the bulk parent material has been previously characterized.

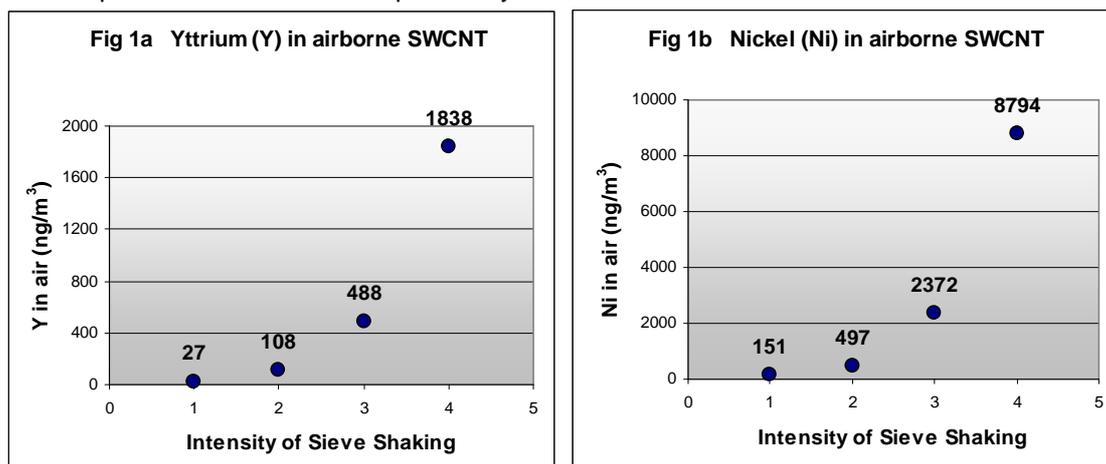


Figure 1: Catalysts such as Yttrium (1a) and Nickel (1b) used in the manufacture of SWCNT serve as useful airborne tracers of process-related emissions. Electrostatic precipitation traps particles from the airstream (300 L/min) into distilled water followed by ICP-MS determination.

N°O2a-3

STRATEGIES FOR RADIOLABELLING OF CARBON NANOPARTICLES

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The employment of radiotracers is a versatile tool for the detection of nano-particulate materials in complex systems such as environmental samples or organisms. With the increasing usage of nanoparticles in applications outside of research laboratories a careful risk assessment of their release into the environment becomes mandatory. However the monitoring of nanoparticles in such complex natural systems as geological formations or ground water is nearly impossible using conventional methods, especially at environmentally relevant concentrations. This obstacle can be overcome by radiolabelling, which may be of crucial value in enabling such research. We present here different radiolabelling strategies for carbon nanoparticles, in particular carbon nanotubes (CNTs) whose intriguing physical properties predestine them for widespread application, so that future release into the environment is to be expected.

We have developed three different approaches for the radiolabelling of CNTs. The first is the iodination of carbon nanotubes using radioactive iodine, e.g. I-125 or I-131 (Fig. 1 top). Using the Iodogen method known from protein labelling strategies it is possible to radiolabel single- and multi-wall CNTs by binding radioactive iodine on the CNT side wall.

The other strategies involve proton irradiation using a cyclotron and cause the incorporation of radioactive beryllium in between the layers of multi-wall CNTs (Fig. 1 bottom). The first option is to directly activate carbon by high-energy proton irradiation (> 34 MeV), which causes a (p,3d) nuclear reaction creating Be-7, which intercalates between the graphitic layers of the CNT. The second option is to mix the CNTs with a lithium containing compound like LiH and irradiate at a much lower proton energy to create Be-7 via the (p,n) reaction on Li. The recoil of the Be-7 from the nuclear reaction causes the incorporation of the radiotracer into the structure of multi-wall CNTs.

The methods were tested for labeling yield, achievable activity concentration, pH-dependent stability of the labeling and the influence on NP-properties. Data thus obtained enables the selection of a radiolabeling method appropriate for different experimental conditions.

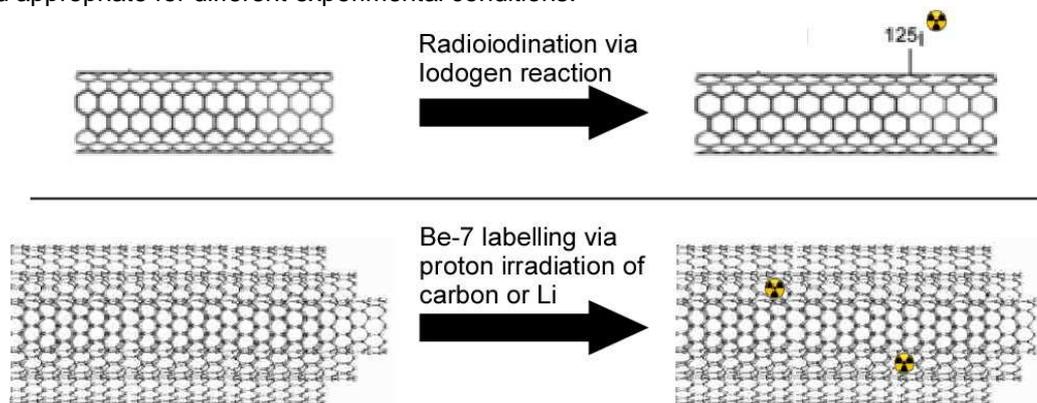


Fig. 1: Radiolabelling strategies for CNTs. Radioiodination using the Iodogen method for radiolabelling single-wall and multi-wall CNTs with I-125 or I-131 (top) and radiolabelling by incorporation of Be-7 in between the layers of multi-wall CNTs, either by direct high-energy proton irradiation or recoil labeling using LiH (bottom)

N° O2a-4

**GENERATION AND CHARACTERIZATION OF DRY NANOPOWDERS AND CARBON NANOTUBES
USING A SHAKER-ATOMIZER TYPE DISPERSER**

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Nanomaterials released during manufacturing or handling processes may lead to worker's exposure and health risks. To protect workers in nanotechnology industry, US NIOSH proposed the recommended exposure limit (REL) for carbon nanotubes and carbon nanofibers, fine TiO₂ and ultrafine TiO₂ particles as 7 µg/m³, 2.4 mg/m³ and 0.3 mg/m³, respectively. In vivo and in vitro toxicity tests are important for assessing the health hazards of nanomaterials which need nanoparticles dispersed directly from dry nanomaterials.

To improve the current methods to disperse dry nanopowders and carbon nanotubes for nanotoxicity researches, a shaker-atomizer type disperser was developed and tested in this study. The disperser consists of shaker, a nanopowder container and an atomizer. The container contains 15 gram of stainless steel balls of 3 mm in diameter to help disperse nanopowders and CNTs and the atomizer further disintegrates agglomerates into smaller nanoparticles. The tested material in this study includes 1 gram nano-TiO₂ powders (AEROXIDE® TiO₂ P25, Degussa Co., Germany) or 0.24 gram carbon nanotubes (XNM-LP-560000, XinNano Materials, Inc., Taoyuan, Taiwan)

Experimental results shows, the nano-TiO₂ particle number concentrations generated by the disperser ranged from $2.56 \times 10^5 \pm 2.63 \times 10^4$ #/cm³ and maintain for at least 2 hrs. The NMD was kept constant at 93 nm. The stability of dispersed nano-TiO₂ concentration is still being improved by introducing dispersion air at the based of the container. A Semi-continuous OC-EC field analyzer (Model 4, Sunset Laboratory Inc., Tigard, OR, USA) employing the Thermal-optical transmittance (TOT) method was used to analyze the composition of dispersed carbon nanotubes. It was found the percentage of element carbon in carbon nanotubes was 93.16±3.47 %, which is close to 95% reported by the supplier.

The morphology of CNTs dispersed by the disperser, size-classified by the differential mobility analyzer (DMA) was observed by the TEM after monodisperse CNTs was sampled directly on TEM grids by the home-made micro-orifice-based concentrated nanoparticle sampler (CNS). The results showed that the average diameter of a single carbon nanotube was 8±2 nm, which is very close to the data of 10 nm reported by the supplier. The morphology of the dispersed CNT agglomerates and stability of CNT number concentrations are being studied and will be reported.

N°O2a-5

**A CARBON NANOTUBES SUSPENSION STUDY BY VORTEX SHAKER:
SAMPLING AND MASS ANALYSIS**

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New materials based on nanotechnology are reaching the market in a wide variety of consumer products. Since these nanomaterials can be in powder form, a suspending powder system is essential for many purposes:

- For in vivo inhalation toxicological studies
- For dustiness evaluation

Different powders suspending systems have been developed like the rotating drum or the single drop. They are used as a reference method to evaluate bulk materials dustiness. Usually cumbersome, these systems need a huge amount of powder (a few hundred grams) and require time-consuming cleaning processes which could be even more tedious when a nanostructured material and/or a toxic powder is used. The vortex shaker is proposed as a solution. Quite easy to handle, this system requires a very small amount of powder (a few grams) in a tube. It has been set up to study the dustiness of any type of powder under controlled and reproducible conditions.

The aim of this work is to aerosolize Multiwalled carbon nanotubes Graphistreght C100 and characterise the generated aerosol. The set up is designed to aerosolize, observe and quantify the carbon nanotubes mass.

In order to observe the generated particles with a Transmission Electronic Microscope giving information on size distribution, particle shape and composition, a TEM grid sampler was operated. This sampler, coupled with a Gilian LFS-113DC pump, allows the aerosol trapping through a copper grid.

In parallel, to measure the aerosol mass, samples have been performed on a 25 mm quartz filter. The carbon nanotubes deposited on the filter are oxidized by combustion at 980°C in the module SSM 5000A (Solid Sample Modul). The melting point is lowered by addition of V₂O₅. Platinum is used as catalyzer. The carbon is then released in CO₂ measured by non dispersive infrared analysis by a Shimadzu detector. To lower the quantification limit, a second infrared detector, the MIR6000 was added in series to the first one. This assembly allows to obtain a limit of quantification of 5 µg / filter.

An hour sampling time was repeated three times at three different shaking speeds.

A continuous mass measurement with TEOM will be added in the set up in order to determine the stability of the aerosols concentrations.

This work is supported by ARKEMA France

*Baron et al 2008 ; Aerosolization of Single-Walled Carbon Nanotubes for an Inhalation Study ;
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N°O2b-1

**STUDY OF NANOPARTICLES LOCALIZATION IN ORGANS AND TISSUES OF RATS AFTER
INTRAPERITONEAL AND INTRAGASTRICAL ADMINISTRATION**

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Study of nanoparticles (NP) localization in organs and tissues of living organisms plays a fundamental role in the characterization of nanomaterials, providing information about effects of NP exposure and their biological action. The data on NP localization in an organism provide a background for the assessment of NP biosafety.

The aim of the work was to study NP localization in organs and tissues of rats exposed to nanoparticles by mean of different analytical techniques. The studies included both the determination of NP concentration in organs and tissues and the confirmation of their presence exactly in nanoscaled form. Two sets of methods for detecting the localization of nanoparticles were used. The first set allowed to detect the presence of the element that represents a nanoparticle (e.g., Ti for TiO₂), or nanoparticle itself in molecular form (C₆₀). It included HPLC with spectrophotometric detection, atomic absorption spectroscopy (AAS), dynamic light scattering (DLS), enzyme-linked immunosorbent assay (ELISA). The second set of methods was presented by microscopic techniques. It allowed to prove a nanoscale form of the defined NP and to assess the functional and morphological state of organs and tissues. We used two types of biological material – slices and homogenates of organs and tissues. For acute (intraperitoneal) and chronic (intra-gastrical) administration to rats carbon NP (fullerene C₆₀), metal NP (Ag of 12 nm average diameter) and metal oxide NP (TiO₂ of 40 nm average diameter), recommended by OECD, were used. The most effective techniques for pretreatment of organs and tissues before NP measurement were selected. Experiments on determination of NP toxic doses (with regard to their specificity and size) for laboratory animals showed that for intra-gastrical administration the values of lethal doses exceed the threshold of 5000 mg/kg.

The proposed methods were validated as tools for quantitative characteristics of NP distribution in the organs and tissues of the exposed rats. Determination of NP localization depending on dose and duration of exposure in terms of acute and chronic experiments was carried out. It was shown that HPLC or HPLC-MS approaches were the optimal techniques to determine the presence of C₆₀ in biological material due to their high sensitivity and accuracy. To determine Ag and TiO₂ NP we have selected AAS. The entire set of methods (HPLC, AAS, ELISA, and microscopies) allowed obtaining data on the distribution of NP in organs, tissues and biological fluids.

Throughout the whole period of observation, no animal death and manifestations of clinical signs of toxicity were observed. The autopsy of animals revealed no visible macroscopic changes in the internal organs. Under acute and chronic experiments, NP penetrated and accumulated in various organs and tissues – lungs, liver, kidneys, blood, brain, spleen, lymph nodes, intestines, etc. Both rutile and anatase forms of TiO₂ NP were found not only in enterocytes of the intestine but also in organs distant from the place of NP introduction (lymph nodes, kidneys, liver, spleen). Revealed TiO₂ were located in the cell cytoplasm in the forms of single nanoparticles, small and large aggregates. Regularities on Ag NP distribution in organs and differences of their shape and size were studied and compared for different administered doses. In all organs examined except the brain and ovary, large single Ag particles (50-200 nm) and its aggregates were detected. Small crystalline Ag NP (10-20 nm) were detected in spleen and kidneys. Large clusters of Ag particles and aggregates were located in liver. Fullerene C₆₀ formed crystals in organs and accumulated in them as large clusters. Giant C₆₀ clusters were found in the mesentery intraperitoneal, spleen and liver (organs of detoxification and immunity). Fullerene was detected in cells inside the endosome, in fat droplets and invaginations of the nucleus.

The methodology developed can be considered as a useful tool for characterization of localization of various nanoparticles in living organisms. The work was supported by the Federal Target Program "Development of nanoidustry infrastructure in the Russian Federation in 2008–2011" and the MARINA project (contract No 236215) of the Seventh Framework Programme of EC.

N°O2b-2

**RISK ASSESSMENT OF RELEASED CELLULOSE NANOCRYSTALS–
MIMICKING INHALATORY EXPOSURE**

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Cellulose nanocrystals (CNCs, also known as nanowhiskers or nanofibers), derived from a variety of natural resources, have received increased attention due to their outstanding chemical and material properties that may be useful for a wide variety of applications. Their exceedingly high strength and stiffness, low cost and high abundance has led to heightened investigation towards the optimisation of their extraction processes, physico-chemical properties and integration within polymer composites¹. Furthermore, CNCs pose a potential alternative to nanofibers with comparable features (*i.e.* carbon nanotubes), that have been shown to elicit adverse health effects². Throughout their life-cycle, from their extraction, to their integration in the production of the nanocomposite, and up to the application in building materials, CNCs may undergo severe abrasion due to withering, drilling and sanding. It is therefore inevitable that CNCs, once embedded into composites, may be released into the environment and most likely could become airborne displaying a potential occupational and accidental risk towards humans via inhalation.

A central problem in gaining an insight into how CNCs may elicit a potentially adverse effect towards human health is the lack of reliable and sophisticated models that may administer realistic doses to biologically relevant *in vitro* systems, and thus mimic exposure *in situ*. For a thorough evaluation of a possible risk to human health a close physico-chemical characterisation of the nanomaterial *in situ* is of utmost importance. The aim of this study therefore, was to establish a method that allows the nebulisation of high aspect ratio nanomaterials such as CNCs. This was achieved using an air liquid interface cell exposure system (ALICE), which has previously only been associated with studies of spherical nanoparticles³.

Bare cotton (bc) CNCs suspended in ultrapure water were characterised after 4hrs of sonication for their dimensions, chemical content and colloidal stability. At a stock concentration of 1mg/mL, transmission electron microscopy (TEM) showed bcCNCs to have a length of 168±72nm and a width of 19±7nm, which is in agreement with the literature⁴. Working suspensions of 1, 0.5 and 0.1mg/mL were then nebulised using the ALICE; this resulted in a deposited dose of 1.19±0.28, 0.66±0.10 and 0.04±0.04µg/cm² respectively, as determined by a quartz crystal microbalance and verified by a chemical detection method. Incorporating state-of-the-art microscopy techniques (TEM and laser confocal scanning microscopy) deposited bcCNC droplets of all nebulised working suspensions showed to have uniform bimodal size distribution with average maximum diameters of 49.03±8.18µm and 5.63±0.41µm). Titration of ion content and observation of the resulting cloud formation as well as deposition characteristics to substantiate the systems' established and reliable functioning is currently under investigation.

The next step is to introduce CNCs derived from other natural resources into the system and study their impact on the nebulisation properties in order to gain a length dependent perspective for the future assessment of the potential biological impact of CNCs.

This study is supported by the National Research Programme 64 "Opportunities and Risks of Nanomaterials" of the Swiss National Science Foundation.

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² "Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study", C.A. Poland, et al., *Nat Nanotechnol.* **2008**, 3(7): 423-428.

³ "A dose-controlled system for air-liquid interface cell exposure and application to zinc oxide nanoparticles", A.G. Lenz, et al., *Part Fibre Toxicol.* **2009**, 6: 32.

⁴ "Cellulose Nanocrystals: Chemistry, Self-Assembly, and Applications" Habibi, Y., et al., *Chem. Rev.* **2010** 110(6): 3479-3500.

N°O2b-3

COLLOIDALLY STABLE, POLYMER ENCAPSULATED QUANTUM DOTS FOR BIOLOGICAL APPLICATIONS

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The unique optical properties of Quantum Dots have attracted a great interest to use these nanomaterials in diverse biological applications. The synthesis of QDs by methods from the literature allows to obtain nanocrystals coated by hydrophobic alkyl coordinating ligands and soluble in most of the cases in organic solvents. The ideal biocompatible QD must be homogeneously dispersed and colloidally stable in aqueous solvents, exhibit pH and salt stability, show low levels of nonspecific binding to biological components, maintain a high quantum yield, and have a small hydrodynamic diameter. To disperse QDs in aqueous solvents it is necessary to manipulate the surface chemistry, and three main strategies have evolved during the past few years: i) ligand exchange, ii) silica encapsulation and iii) polymer encapsulation.

Polymer encapsulation represents an excellent scaffold on which to build additional biological function, allowing for a wide range of grafting approaches for biological ligands [1]. Our investigations suggest that with ligand exchange methods the QD photoluminescence is often compromised and that the growth of a thin, uniform silica shell that encapsulates a single QD is at present nontrivial. We have shown that the commonly synthesized QD in organic solvents transfer well using a poly(styrene-co-maleic anhydride) polymer and that the resulting biocompatible QD is homogeneously dispersed and colloidally stable in aqueous solvents, exhibits pH and salt stability and maintains a high quantum yield.

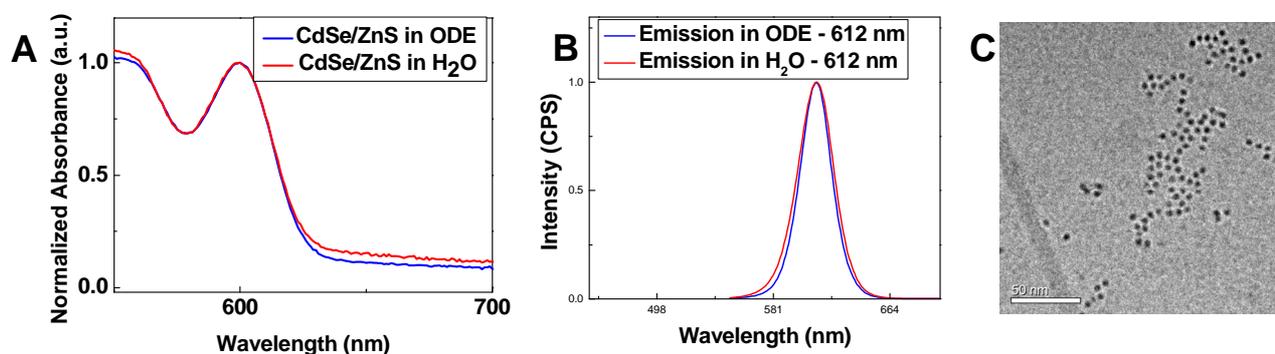


Figure 1: (A) UV-vis absorption spectra, (B) fluorescence spectra of QDs before (in 1-octadecene) and after phase transfer and (C) TEM image of QDs after phase transfer.

As the polymer encapsulated QDs were stable prepared in aqueous solution, and are cleaned with water, we did not expect any significant issues in terms of their dispersion. Our studies confirmed these hypotheses as repeated centrifugation and resuspension cycles did not result in any significant aggregation issues, as observed by UV-vis spectroscopy and DLS.

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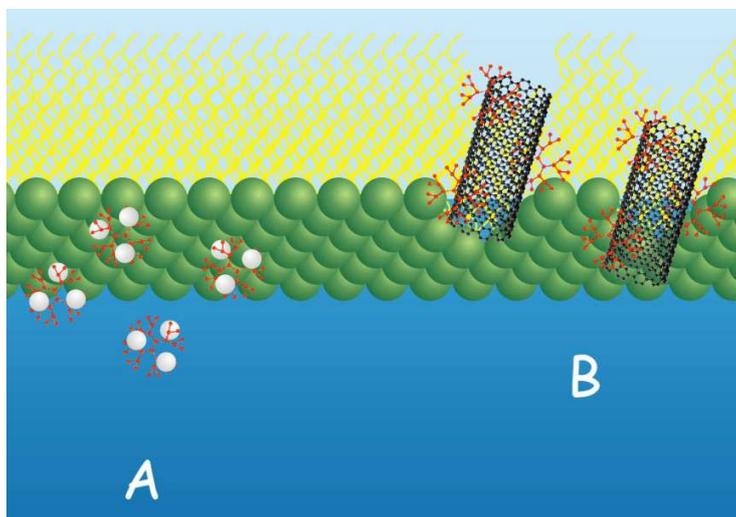
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N°O2b-4

MEMBRANE MODEL AS A NEW METHODOLOGY FOR NANOTOXICOLOGY INVESTIGATION

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Nanomedicine increased rapidly with nanotechnology over the last decade, especially with the development and application of nanomaterials in different areas. These include molecular imaging systems, diagnostics, drug delivery, and biosensors, as well as products such as antibacterial creams and medical surgical instruments. Despite the rapid progress and acceptance of nanomedicine, the potential effects of nanomaterials in biological systems due to prolonged exposure at various levels of concentration, has not been established. In order to promote the safe development of nanomedicine, it is essential to evaluate the adverse health consequences. Due to the complexity of the mechanisms involved in the interactions between nanomaterials and biological systems, biophysical aspects are difficult to be investigated, especially in natural samples and in real time. In this study we introduce a methodology to evaluate the interactions between nanoparticles and phospholipid biomembrane models, using the Langmuir technique. Our main goal was to elucidate the biophysics interaction of nanomaterials in cell membranes, at the molecular level. The penetration of nanoparticles into cardiopin (CLP) monolayers was studied using spectroscopy frequency generation (SFG) and also by kinetics absorption and surface pressure measurements. Incorporation of the nanomaterial into the monolayer was pronounced, as revealed by the shift in the molecular area to higher values, compared to the values obtained for pure CLP. SFG was used for the first time for nanotoxicology studies. SFG is a technique with surface specificity and chemical selectivity capable of obtaining vibrational spectra of species at surface and interface. SFG spectroscopy was used to investigate the CH stretches of alkyl chain (hydrophobic tail of CLP) and the OH stretches of water molecules interacting with the Langmuir. Such investigations may be of great importance to understanding the toxicity of nanomaterials at the molecular scale and bringing important benefits to the nanomedicine development.



*Schematic representation of the nanomaterials interaction with the lipid monolayer surface. The interactions may occur between the nanomaterials and the lipid membrane in two different directions: (A) adsorption via electrostatic interaction, and (B) incorporation across the membrane. Adapted from Cancino, J.; Nobre, T.M.; Oliveira, O.N.; Machado, S.A.S.; Zucolotto, V. A new strategy to investigate the toxicity of nanomaterials using Langmuir monolayers as membrane models. *Nanotoxicology* 2011, 1-10, In press.*

N°O2b-5

**ENGINEERED NANOMATERIAL QUANTIFICATION IN COMPLEX MATRICES:
PIXE CASE STUDIES**

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With the advent of the discovery of engineered nanomaterials (ENMs) and their fundamental different properties than bulk materials, there has been a rush in the industry to produce and use these new materials in different products, touching a wide area from semiconductors, medicine, products of personal use, and food. There are health concerns regarding the use of ENMs. The size of ENMs coupled to the complex biological matrices they can be surrounded (i.e. organs, cell culture media, food, etc.) makes it difficult to provide a proper quantification. PIXE (Particle-Induced X-ray Emission) is a competitive technique able to address the challenge of high sensitivity ENM quantification (ppm levels) in complex matrices both solid and liquid.

The use of PIXE is presented in three different case studies: 1) the biopersistence of SiC and TiC nanoparticles in lungs; 2) the biodistribution of ingested SiC measured through feces excretion and other organs where it was found that while given dose was not toxic the organ composition was affected; and, 3) the quantification of ENMs in liquids through our non-vacuum measurements setup. These case studies highlight the potential of PIXE as a technique to quantify both ENMs and their surrounding complex matrices with a minimal sample conditioning.

N°O2c-1

**MEASURING PARTICLE SIZE DISTRIBUTIONS OF NANOPARTICLES IN AQUEOUS MEDIA USING
FFF AND LIBD**

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In recent decades the terms nanotechnology and nanoparticles became very popular. Nanotechnology is considered as one of the key technologies of this century with a huge economic potential, and the number of products containing nanoparticles is drastically increasing. Engineered nanoparticles (ENP) are defined as particles with a diameter <100nm which are designed to have specific properties. ENP are used in many industrial sectors and products, such as paints, drugs, cosmetics, semiconductors, catalysts, environmental technology and material research. The increasing production and use of nanoparticles will eventually lead to their release into the aquatic environment. Despite the rapid increase of ENP production volumes analytical techniques capable of detecting and monitoring individual nanoscale particles (including ENP) in complex (natural) aqueous matrices, such as wastewater effluents or surface waters are currently lacking.

Laser-induced breakdown detection (LIBD) is a method capable of detecting nanoscale particles in aqueous matrices. The measurement principle is based on the detection of a plasma selectively generated on colloids that are hit by a focused, pulsed laser beam.

An average number and size of nanoscale particles in suspension can be derived from so-called energy curves which are obtained by a stepwise increase of the laser energy and recording the breakdown probability (BDP) - referring to the ratio of the number of plasma events detected to the total amount of laser pulses - at every energy step. The energy curves can be described by a semi-empirical model [1] with two free parameters, one of them referring to the size, the other to the concentration. Using more sophisticated evaluation algorithms even quantitative, polydisperse size distributions can be derived instead of average size and concentrations. However, for that purpose, the energy curves have to be recorded with very high energy resolution requiring up to 1h of measurement time. In order to reduce the measurement time, the polydispersity of the samples have to be reduced, which can be achieved using a flow field flow fractionation (FFF) device. In the FFF particles are size-fractionated in a ribbon-like channel and based on the retention time in the channel, the size of the particles can be estimated. With prior knowledge of the particle size, the number concentration can be determined within a few seconds using the LIBD. In this study we coupled the LIBD to the FFF and recorded the BDP of the effluent of the FFF at a fixed laser energy. First results obtained from 100nm particles that were fractionated in the FFF and then measured in-line with the LIBD revealed a clear correlation between the BDP (recorded at fixed laser energy) and the particle concentration in suspension. Further experiments will be conducted using polydisperse suspensions to determine the figures of merit of this promising approach.

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N°O2c-2

LAB-ON A CHIP *uTAS* (MICRO TOTAL ANALYSIS SYSTEMS) FOR THE HIGH-THROUGHPUT MEASUREMENT OF NANOMATERIAL SOLUBILITY

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One general consensus in the nanotoxicology community has been the lack of suitable and convenient methods, particularly tools that can follow the dynamics of nanomaterial when in a complex medium. What is required is a reliable high-throughput device capable of mass screening; such a research approach will undoubtedly coincide with the goal of industry and regulation. Here, we present the concept of *uTAS* (Micro Total Analysis Systems), a term coined in the 1990s to encapsulate the integration of laboratory process in one chip and the potential on the use of such microchip technology in nanotoxicological based investigations. Our focus here is on the measurement of ZnO dissolution using commercially available microchip electrophoresis with conductivity detection that envelops the concept of *uTAS* technology. We have show that this technique is suitable for the measurement of $[Zn^{2+}]$ in an ecotox relevant medium, like fish medium, with analysis time of less than two minutes. In addition to its potential as high-throughput platform, the power of the instrument lies in its capability to accurately distinguish particulate zinc vs. ionic zinc; this is achieved through the on-chip capillary electrophoresis separation module fabricated into the microchip. Our results clearly show promise on the use of such *uTAS* technologies but clearly several issues will need to be addressed before such a measurement tool can be shown that it is indeed fit-for-purpose.

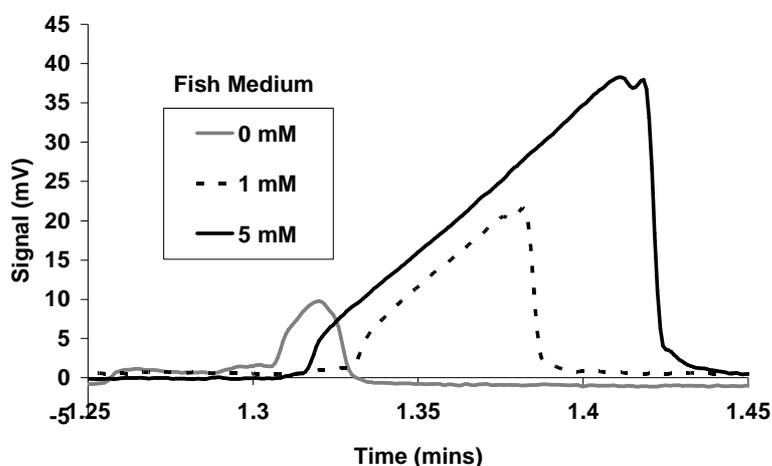


Figure 1. Electropherogram for Zn^{2+} (at various concentrations) when in fish medium.

N°O2c-3

**SPECIES SELECTIVE PRE-CONCENTRATION AND QUANTIFICATION OF AG, AU AND PD
NANOPARTICLES USING CLOUD POINT EXTRACTION AND GRAPHITE FURNACE ATOMIC
ABSORPTION SPECTROMETRY**

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The production and application of metallic nanoparticles (MNP) for consumer products, for catalysis, and in the medical field has been rapidly growing during the past decade. Concerns regarding safety and toxicity of these materials have, however, been risen. Several studies showed the toxicity of nanoparticulate silver (Ag-NP), gold (Au-NP), palladium (Pd-NP) in vivo and in vitro. Also, the adverse effects of MNP exceeded significantly those from ionic species of the same metal. Hence, methods that assess the environmental exposure must not only give the overall concentration for an element but also allow species selective determination of MNP. Another crucial issue is the quantification in complex matrices where MNP are most likely to be found, such as waste water treatment plant (WWTP) effluent.

Several techniques like solvent extraction, field-flow fractionation, single particle inductively coupled plasma mass spectrometry (ICP-MS), or cloud point extraction (CPE) have been suggested to meet the above criteria. The latter method uses the characteristic formation of micelles in aqueous solutions of surfactants when heated above a certain temperature termed cloud point. The micelles will segregate and can be used for the extraction and pre-concentration of trace compounds. Previous studies used CPE for MNP only in combination with ICP-MS which required sample digestion prior to quantification and thus makes the method labour intensive and increases the limit of detection due to the necessary dilution up to 100-fold.

We present a method for the quantification of Ag-NP, Au-NP, and Pd-NP with CPE using the surfactant Trion X-114 and electro-thermal atomic absorption spectrometry (ET-AAS). Here the pre-concentrated sample can be introduced in to the measurement apparatus directly. CPE conditions and the use of additives were optimized for each metal to gain species selective extraction and high recovery (> 90 %) for MNPs. After extraction the surfactant-rich phase (~500 µL) was dissolved in a small amount (100 – 200 µL) of ethanol to reduce viscosity. Using optimized GF-AAS conditions and additives for each species, detection limits of 1 (Ag-NP), 7 (Au-NP) and 70 (Pd-NP) ng·L⁻¹ in a 40 mL water sample were achieved. At the same time the extraction of ionic metal of the same element was below 2 % in all cases. For method validation differently coated nanoparticles of each species were subjected CPE. No effect of the coating on extraction efficiency was observed. Tolerance on organic matter was evaluated using commercially available humic acid (HA). No significant effect on the efficiency was observed for HA concentrations up to 10 mg·L⁻¹. Spiking experiments in real water samples (tap, river and WWTP effluent water) yielded in a recovery of more than 90 %, suggesting that our method can be readily applied for the quantification of Ag-, Au- and Pd-NP in environmental samples.

In the next step we want to develop methods for determining the MNP-size distribution in environmental samples after CPE. Preliminary experiments using transmission electron microscopy (TEM) showed that particle size is not affected by our method.

N°O2c-4

QUANTITATIVE ANALYSIS OF ENGINEERED NANOPARTICLES IN FOOD AND ENVIRONMENT

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Consumer products containing engineered nanoparticles (ENPs) are already on the market. During the production, use and disposal/recycling of these products ENPs might expose consumers and may be released into the environment. For regulation enforcement and risk assessment regarding, validated quantitative methods for ENP analysis in a wide variety of matrices are needed. These methods are not available so far.

The analytical strategy to be adopted depends strongly on the information requested, the type of the ENPs and the nature of the matrix. E.g. samples with a complex matrix require in most cases a pre-treatment in preparation of analysis in order to isolate the ENPs from the matrix. The isolated particles have to be stabilized and pre-concentrated prior to further particle characterization. The following analysis aims at the determination of particle size distribution and selective element & mass concentration.

Field Flow Fractionation (FFF) is one of the most promising analytical techniques for these tasks. FFF is an analytical particle sizing and separation technique coupled to specific detectors like multi-angle light scattering, UV-VIS spectrometry and ICP-MS for quantification of particle size distribution as well as size and element specific mass concentrations.

The sample preparation for FFF must provide the isolation of ENPs from the matrices. This can be achieved by specific approaches which are carefully adapted to the properties of particle (SiO₂ and Ag) and matrix. Colloidal extraction, acidic, alkaline and enzymatic attack was investigated in detail. The effect of the sample preparation on particle size distribution and elemental mass recovery was studied and optimized.

To establish the required robust FFF methods for analyzing the isolated ENPs a systematic approach to thoroughly optimize and validate FFF for certain combinations of ENPs and sample matrix has been adopted. Critical system parameters like cross flow rate, carrier composition, membrane type, channel height and injection volume, mass and procedure have been investigated for their influence on the accuracy and overall performance of FFF. Similar systematic studies were performed for the specific particle detection following separation as e.g. by light scattering and ICP-MS. Using this systematic and stepwise approach we arrived at optimized run conditions for the FFF and for the specific particle detection (ICP-MS). The optimization schemes aimed at maximum recovery, lowest possible influence on particle size distribution, minimum alteration of the original ENPs during analytical procedure and practical applicability of the method (e.g. analysis time).

Using these analytical techniques we systematically developed methods to detect and quantify ENPs in complex food and soil/sewage sludge matrices. Examples of silica ENPs in tomato soup and silver-ENPs chicken meat and soil/sewage sludge will be presented. The results show that the tested ENPs can be analyzed in complex matrices as soup or meat with good precision and mass recovery (> 80%). We could demonstrate that with our systematic approach optimized analytical tools for ENP analysis and characterization in complex matrices as food and environmental media can be developed. These methods are considered to be compulsory for future tasks including enforcement of future regulations, risk assessment and toxicology.

N°O2c-5

DETECTION OF NANOPARTICLE HEAVY METAL POLLUTANTS IN WATER BY LASER-INDUCED BREAKDOWN SPECTROSCOPY (LIBS)

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Pollution of air and water are major concerns both at domestic and European Union level. With the aim in view to preserve and improve water quality, the European Community has adopted a water framework directive (WFD). Its objective is to achieve a good ecological and chemical status of waters by 2015. In this context, research in metrology has been engaged at INERIS in order to detect pollutants such as heavy metals {both in ionic or particulate forms}. Though they were not cited in the directive since they are considered as emerging pollutants, nanoparticles are an object of concern whose detection should be made possible as well. The constraints imposed by regulations and the emergence of new pollutants, emphasize the need to develop an instrument allowing in-situ and real-time elemental identification and mass concentration determination of particles suspended in water. Environmental monitoring and surveillance of pollutants in particulate form possibly released in waste waters by industries involved in nanoparticle manufacturing and processing are two telling examples of applications of such instrumental development. The LIBS (Laser-Induced Breakdown Spectroscopy) technique was retained to tackle such issue.

LIBS consists in focusing a powerful laser pulse on a material (solid, liquid, gas, aerosol, nanoparticle flow) whose elemental composition is to be determined. The strong heating of the sample at the focusing spot leads to the ignition of a hot and luminous transient ionized gas called plasma. Plasma light contains the signature of all the chemical elements the interrogated material is made of. This signature is read by sending the emitted light through a spectrometer equipped with a detector. The LIBS signal presents itself as an optical emission spectroscopy spectrum displaying lines corresponding to the detected elements. The features of LIBS (all optical technique, fast, non-intrusive, no sample preparation, remote and stand-off analysis possible) do make it a promising analytical chemistry technique intended to be operated at industrial or environmental sites. Current elemental analysis techniques require time consuming procedures involving several steps. Skilled personnel are sent to the sites where samples are to be picked up. Back to the laboratory, these are prepared prior to the analysis itself. The LIBS systems developed at INERIS are intended to be operated on-site, in automatic mode on the opposite, without manual intervention except for maintenance.

A LIBS system has been coupled with a device allowing sampling of water. The liquid sample containing particles is flowed through a flexible tube connected to a capillary. The liquid is then ejected from the latter in the form of a liquid jet. Powerful laser pulses are focused on the liquid jet, thereby generating a plasma for the elemental analysis as explained above. Continuous monitoring of liquid samples is made possible using such configuration. The LIBS response was then studied as a function of several experimental parameters such as laser wavelength, laser energy, laser - liquid jet surface interaction, particle sizes etc... The experiments were carried out using micrometric and submicrometric-sized particles. Particle suspensions were controlled using laser diffraction and dynamic light scattering devices. It was found that the LIBS response does contain two components whose dependence on the above parameters was studied. The particle sampling rate (PSR) reflects the stochastic nature of the detection of the particles suspended in liquid using a laser-induced plasma, the sampling volume of which being not continuous neither in space nor in time. The greater the PSR, the larger the amount of particles sampled by the laser-induced plasma. The signal-to-noise ratio (SNR) represents the signal quality. Optimizing these two components comes down to maximizing the LIBS response. A first series of experiments allowed to find out the parameters optimizing the analysis on the liquid jet and the performance of the system in its current configuration. A sampling method other than the liquid jet is envisaged to be tested as well.

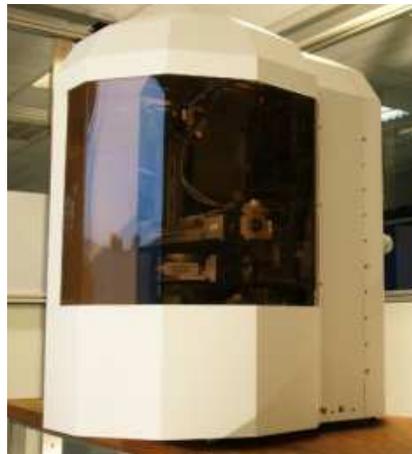
N°O2d-1

NANOBADGE, A TOOL FOR ENGINEERED NANOPARTICLES EXPOSURE ASSESSMENT

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An important safety aspect of the workplace environment concerns the severity of its air pollution with particles in the 10 μm - 10 nm size range including nanoparticles and their aggregates. Depending on the size and chemical nature of engineered Nanoparticles, exposure to those through inhalation can be hazardous because of their intrinsic ability to reach and deposit in the deep alveolar region of the lung. When the hazards are uncertain, precautionary safety measures in the nanomaterial industry should ideally involve a continuous monitoring of the personal exposure to airborne nanoparticles and their aggregates in order to minimize judiciously the exposure and to assess the effectiveness of implemented measures. Current instruments and methods are expensive, time-consuming and require specialized skills.

This paper describes the principle and operation of the NanoBadge tool. Nanoparticles are collected by air sampling on filters with a proprietary technology. Filters are then analyzed automatically by X-ray fluorescence spectroscopy yielding the elemental composition of the collected material and their averaged concentration in air. The measure of the engineered nanoparticles concentration by their constitutive element represents a very powerful strategy, because it is a way to get rid of the existing high and fluctuating background level of natural and anthropic nanoparticles. The low limits of detection obtained by this method are very promising. Conclusions about the ability and performances of this monitoring technique to perform personal exposure measurements and to detect unusual levels of nanoparticles and their aggregates can be drawn.



NanoBadge sampler and X-ray fluorescence spectrometer

This work was supported by NanoBadge, Alcen Group. CEA worked in collaboration with Irelec and ID3 semiconductors for devices development.

N°O2d-2

MEASUREMENT METHODS FOR THE EC DEFINITION OF NANOMATERIALS

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Nanoparticles are already used in several consumer products including food, food packaging and cosmetics and their detection and measurement in complex matrices, such as food and cosmetics, represent a particularly difficult challenge. In order to fill the void in the official definition of what constitutes a nanomaterial the European Commission has published in October 2011 its recommendation on the definition of nanomaterial. This will have an impact in many different areas of legislation, such as the European Cosmetic Products Regulation

The text of the recommendation calls for the measurement of the number based particle size distribution in the 1-100 nm size range of all the primary particles present in the sample independently of whether they are in a free, unbound state or as part of an aggregate/agglomerate.

This recommendation, while being very comprehensive in describing the criteria for classifying whether a material should be considered “nano”, does present great technical challenges for those who must develop valid and compatible measuring methods¹.

In this talk I will give a brief overview of the current state of the art for the size measurement of particles in the submicron range and I will present some of our most recent results on the combination of flow field flow fractionation with multi detector analysis showing a possible way forward for solving this challenging analytical problem.

¹ *Measuring nanoparticles size distribution in food and consumer products: a review*
L. Calzolai*, D. Gilliland and F. Rossi. *Food Additives and Contaminants*. 2012, 1–11, *iFirst*

N°O2d-3

NANODEVICE - NOVEL CONCEPTS, METHODS, AND TECHNOLOGIES FOR THE PRODUCTION OF PORTABLE, EASY-TO-USE DEVICES FOR THE MEASUREMENT AND ANALYSIS OF AIRBORNE ENGINEERED NANOPARTICLES IN WORKPLACE AIR

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NANODEVICE is a research project funded by the European Commission in the context of the 7th Framework Program. The duration is 48 months starting 1st of April 2009 and ends in March 2013.

Due to their unique properties, engineered nanoparticles (ENP) are now used for a myriad of novel applications with great economic and technological importance. However, some of these properties, especially their surface reactivity, have raised health concerns, which have prompted scientists, regulators, and industry to seek consensus protocols for the safe production and use of the different forms of ENP.

There is currently a shortage of field-worthy, cost-effective ways - especially in real time - for reliable assessment of exposure levels to ENP in workplace air. In addition to the problems with the size distribution, a major uncertainty in the safety assessment of airborne ENP arises from the lack of knowledge of their physical and chemical properties, and the levels of exposure. A special challenge of ENP monitoring is to separate ubiquitous background nanoparticles from different sources from the ENP.

To meet these challenges, NANODEVICE provides new information on the physico-chemical properties of engineered nanoparticles (ENP) and information about their toxicology. The main project goal is to develop innovative concepts and reliable methods for characterizing ENP in workplace air with novel, portable and easy-to-use devices suitable for workplaces. Several novel measuring devices and samplers are currently tested in the field to assess their performance regarding exposure measurements to ENP's from workplace air. During a free international seminar held at NANOSAFE 2012, attendees will learn about the newly developed devices. The purpose of the project is also to promote the safe use of ENP through the international nanosafety forum SENN2012 (www.ttl.fi/senn2012). The research leading to these results has received funding from the European Commission under grant agreement FP7-211464-2 (www.nano-device.eu).

N°O2d-4

**DETECTION OF NANOPARTICLE AGGLOMERATES TRAPPED IN A LOW PRESSURE RF
(RADIO-FREQUENCY) PLASMA DISCHARGE USING LIBS (LASER-INDUCED BREAKDOWN
SPECTROSCOPY)**

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Nanotechnology is often said to be the industry of the 21st century. In most cases, the designing of nanostructured materials implies utilizing engineered nanoparticles as basic building blocks. Such materials often have new functionalities and enhanced properties which make them of great interest for many industrial applications. The apparent limitless possibilities offered by nanotechnologies in terms of applications and economic gain are to lead to a dramatic increase of engineered nanoparticle production. The need for nanoparticle-based materials is therefore expected to grow along with that for nanometrology allowing characterization of nanoparticles in-situ and in real time if possible. Workplace surveillance and process control are among many others issues where such instrumental development is required.

The LIBS technique was deemed as a promising tool to deal with such issues [1-3]. LIBS consists in focusing a powerful laser pulse on a material (solid, liquid, gas, nanoparticle flow) whose elemental composition is to be determined. The strong heating of the sample at the focusing spot leads to the ignition of a hot and luminous transient ionized gas called plasma. Plasma light analyzed through optical emission spectroscopy contains the signature of all the chemical elements the interrogated material is made of. Simultaneous detection of all the elements and the determination of the corresponding mass concentration are possible at various pressure conditions. Being all optical, it is not intrusive and remote or stand-off analyses are even possible. Besides, samples do not need preparation. The aforementioned features do make LIBS a promising analytical chemistry method intended to be operated at industrial sites.

With the final aim in view to enhance detection of aerosols, preliminary experiments were carried out coupling the LIBS analyzer with a low pressure RF (Radio Frequency) plasma discharge acting as a particle trap [4,5]. Agglomerates of composite nanoparticles of SiC and Al₂O₃ were injected into the RF plasma discharge where they were trapped and remained in suspension. Nanosecond laser pulses were then focused on the trapped particles which resulted in their vaporization, thereby producing the LIBS signal. LIBS detection was therefore achieved with a satisfying signal to noise ratio at a reduced pressure of 0.25 mBar instead of the atmospheric pressure. Thus, this coupling presents several advantages. All injected particles are trapped and can potentially be analyzed, thereby improving sampling. Organic particle analysis is made possible without interferences with the C, H, N, O, elements already present in the air when using an inert gas such as argon or helium for RF plasma generation. In addition, the signal-to-noise ratio is expected to be better at reduced pressure than at atmospheric pressure.

The obtained results, though qualitative so far, have demonstrated the potentialities of such coupling for elemental identification of the elements composite nanoparticle agglomerate are made of. Future experiments are intended to optimize the LIBS detection, to assess the detection limits and to obtain quantitative results when tackling issues such as process control or ambient air monitoring. The use of other laser diagnostics could be envisaged as well.

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[5] Deposited patent entitled "Particle detection technique using an RF plasma discharge and LIBS"

N°O2d-5

CHARACTERIZATION OF MANUFACTURED TiO₂ NANOPARTICLES

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The technological advances in nanomaterials during the last twenty years have allowed the development of new applications in various fields such as medicine (for therapeutic and diagnostic purposes), environment (water treatment), food (membranes for purification), industry (electronics, energy)...

The scientific knowledge on health and environmental risks associated to nanoparticles shows that airborne nanoparticles in ambient air have potential adverse effects on the health of exposed workers and general population (Witschger *et al.*, 2005). Today, only the particles smaller than 2.5 microns are considered and measured by the air quality monitoring networks in France. However, studies have shown that excessive amounts of solid and poorly soluble aerosols are able to penetrate deep into the lungs by inhalation, to stay there permanently and to create an pulmonary overload weakening the ability of body's defense (Witschger *et al.*, 2005; Hervé-Bazin B, 2007). These effects are all the more important since the particles are fine.

In the context of exposed workers, a report by the French Agency for Environmental and Occupational Health Safety (ANSES), entitled "Nanomaterials and safety", indicates a production of several thousand tons of manufactured titanium dioxide (TiO₂) nanoparticles in French industry. Therefore, in order to fulfil its aim of protecting human health, ANSES began to focus on the toxicology of nanoparticles such as the TiO₂ (Vincent, 2008) in order to protect the health of the workers involved in their production process. However, to perform reliable toxicology studies, it is necessary to determine the relevant characteristics of TiO₂ nanoparticles, such as the size, the crystal structure and the count size distribution, because they are directly correlated to human health problems such as respiratory diseases, cardio-vascular troubles.

Consequently, we have developed analytical methods for the characterization of TiO₂ nanoparticles in size, crystal structure (anatase and rutile) and count size distribution, based on different techniques to characterize five different manufactured TiO₂ nanoparticles.

In this study, different measurement techniques have been implemented: X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Scanning Mobility Particle Sizer (SMPS) and Aerodynamic Particle Sizer (APS). The two measuring instruments SMPS (ISO 15900) and APS allow measuring the count size distribution, over a large range between 15 nm to 20 µm, on the dustiness of airborne particles generated with different manufactured TiO₂ powders using with a powder disperser "Naneum aerosoliser" based on the vortex principle.

The TEM and XRD results lead to a relatively good agreement between data from the manufacturer and our characterizations of primary particle size and crystal structure. With regard to the dustiness, the results show a strong presence of agglomerates / aggregates of primary particles. Nevertheless, the results show a significant presence of emitted airborne nanoparticles with a diameter below 100 nm (composed of isolated primary particles and small aggregates / agglomerates formed from a few primary particles): the number proportion of these particles vary from 0 to 44 % in the measurement range 14-360 nm depending on the types of powders and corrections of measurements. This work arises from the OECD WPMN sponsorship programme.

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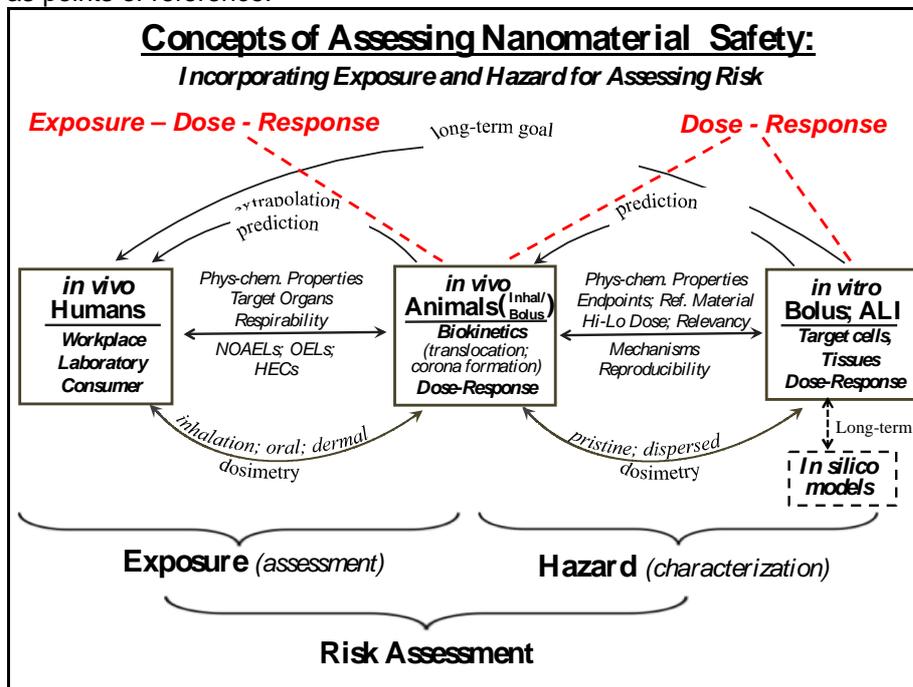
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N°PL3

CONCEPTS OF SAFETY ASSESSMENT OF ENGINEERED NANOMATERIALS (ENM)

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Exposures of humans to nano-sized particles has occurred throughout human history, and it is only with the advent of nanotechnology and its application for diverse industrial, medical and consumer uses that reports about the potential of ENMs causing adverse health effects has raised serious concerns. Such concerns stimulated research in the slowly maturing field of nanotoxicology, resulting in a steadily increasing number of publications showing that engineered nanomaterials – because of their specific physico-chemical properties – can induce significant adverse effects. Although most of these studies were performed using dose-response data of questionable relevance for real-world exposure scenarios, they had led to a widespread perception that essentially all nanomaterials are “toxic” and pose a significant health risk. However, the sole reliance on Dose-Response data falls short of enabling a comprehensive safety assessment of ENM. Little attention appears to be directed at the importance of incorporating in toxicity tests exposure and dosimetry-related information including ENM biokinetics. Such data are necessary in order to not only identify a hazard, but also to characterize a potential risk, which is a fundamental goal of toxicity testing. A practical approach will be discussed to correlate results of *in vitro* and *in vivo* studies and – importantly – emphasize the need of obtaining *in vivo* exposure data so that risk can be assessed once experimental Exposure–Dose–Response relationships in rodents are available. Such relationships will be a basis for the extrapolation of risk to humans using realistic exposure scenarios provided species differences in dosimetry are considered. A long-term goal of ENM toxicity testing is to develop and validate simple *in vitro* tests and perhaps *in silico* approaches whose results are not only useful for hazard identification and hazard ranking but will also allow to perform a quantitative risk assessment and derive “safe” occupational exposure levels. These concepts will be illustrated with results of subchronic rodent inhalation studies of ENM in comparison to benign and reactive reference materials as points of reference.



N°O3a-1

HOW DO OXIDE NANOMATERIAL DISPERSIONS EVOLVE IN AN IN VITRO ASSESSMENT?

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With the advent of the discovery of engineered nanomaterials (ENMs) and their fundamental different properties than bulk materials, there has been a rush in the industry to produce and use these new materials in different products, touching a wide area from semiconductors, medicine, products of personal use, and food. There are health concerns regarding the use of ENMs. It has been shown that the way ENMs are prepared for in vitro tests influences the toxicity results. The heterogeneity observed in the results from the potential toxicity assessment evaluation of nanoparticles, prevents their adequate comparison or evaluation. Several factors are reported to potentially influence the modification of their physicochemical properties during the different stages of the assessment (the pre-dispersion, the mixture with the culture medium and the incubation time). Nevertheless, the extent of the modifications produced by these factors or conditions remains to be adequately described.

In this work, the evolution of some key physicochemical parameters of oxide ENM dispersions was studied using an in vitro biological assessment. A model ENM, TiO₂, was prepared in dispersion, due to its importance in commercial products (i.e. used in cosmetic products) and given that it is part of the OECD list of reference materials. Two cell lines (IHK and A549) were assessed at different incubation times. PIXE (Particle-Induced X-ray Emission) was used to quantify the ENM fractions (supernatant and sediment), and CLS (Centrifuge Liquid Sedimentation) was used to determine the particle size distribution. PIXE is an ion beam technique able to address the challenge of high sensitivity ENM quantification (ppm levels) in complex matrices either solid or liquid. In order to elucidate the contribution of the cell lines to this evolution, the same series of measures were performed only on each culture media (without cell lines). The results will describe the extent of the evolution produced in these key parameters, and the correlation on the used techniques will be discussed. The validity of the current assumption that the given dose equals the effective dose will be discussed in light of the findings.

N°O3a-2

INTERACTION OF FIBRINOGEN AND ALBUMIN WITH TITANIUM NANOPARTICLES OF DIFFERENT CRYSTALLINE PHASES

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TiO₂ nanoparticles (NPs) are widely used for different kinds of industrial products including paints, self-cleaning glasses, sunscreens. TiO₂ is also employed in photocatalysis, e.g. for waste water treatment and the number of applications is expected to increase. Micrometric TiO₂ is generally considered a safe material, but since the fate of NPs within the human body may largely differ from its micrometric counterpart (Oberdörster 2005), studies on the interaction of NPs with endogenous molecules are needed. In particular, the adsorption of proteins on NPs ultimately influences their biological fate. Fibrinogen and albumin are two of the most abundant plasma proteins. Fibrinogen plays different relevant biological functions such as blood clotting, formation of thrombi following vessel injury (Jackson 2007), foreign body reactions following implantation of prosthesis (Thevenot 2008) and in the inflammatory response to NPs (Deng 2011). Albumin is the main transport of solutes in the blood stream to their target organs and also maintains the pH and the osmotic pressure of plasma (Kobayashi 1999).

The aim of this work is the study of the adsorption of fibrinogen and albumin on titania nanoparticles of different crystalline phases (rutile and anatase).

In this study we investigated the interaction of albumin and fibrinogen with TiO₂ samples using an integrated set of techniques. The affinity of each protein for each TiO₂ surface was investigated thanks to the construction of adsorption isotherm curves in which the amount of adsorbed protein is reported against the increasing concentration of free protein upon adsorption. The protein concentration was evaluated spectrophotometrically by using the well-known bicinchoninic acid assay (BCA). The variation of the surface charge of the TiO₂ NP conjugated with the protein with respect to the naked NP was used to indirectly estimate both the surface coverage and the reversibility of the adsorption process. The surface charge was monitored by measuring the ζ potential with a conventional electrophoretic light scattering (ELS) system. The extent of protein deformation was evaluated by Raman Spectroscopy. We found that both proteins adsorb irreversibly against electrostatic repulsion likely allowing conformational changes. However the affinity of fibrinogen for the TiO₂ surface appears higher than that of albumin. The amount of protein adsorbed was found to vary on the different crystalline phases either because of the crystal structure or of the particle size and shape.

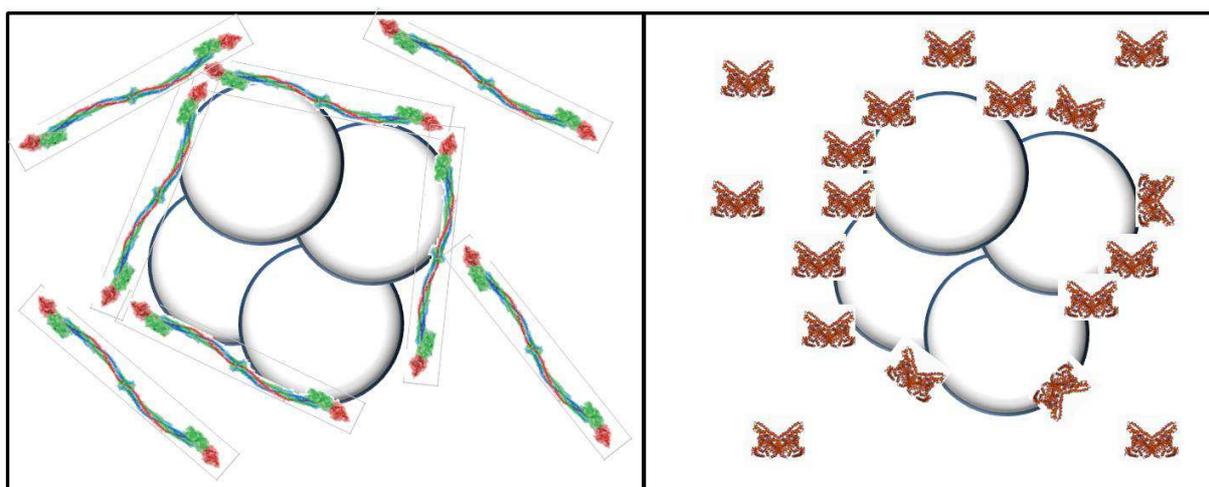


Figure: Proteins adsorption on TiO₂ nanoparticles: fibrinogen (left), albumin (right).

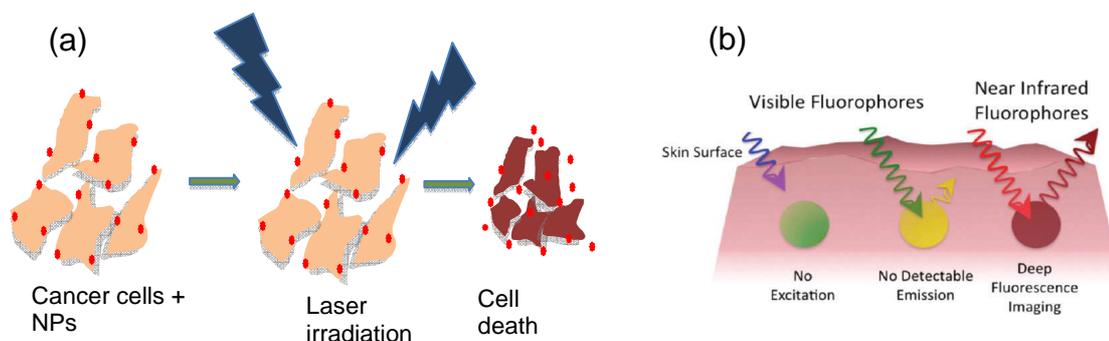
N°O3a-3

AN EFFECTIVE PHOTOTHERMAL THERAPY AGAINST CANCER CELLS AND DEEP TISSUE IMAGING WITH TARGETED NIR QD

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Quantum dots (QDs) have gained greater attention owing to their remarkable photophysical properties such as high fluorescence quantum yield (QY), superior stability against photobleaching, a narrow and symmetric emission spectrum and a broad and continuous excitation spectrum. These characteristic properties make QDs superior fluorescent probes to organic fluorescent dyes and proteins for biomedical imaging applications. Among different imaging modalities, optical imaging techniques have attracted great interest in the diagnostic process owing to their easiness in operation, better temporal resolution, and relative low cost. Since most tissue chromophores, including oxyhemoglobin, deoxyhemoglobin, and melanin, exhibit comparatively weak absorbance in the near infrared (NIR) spectral range (700– 900 nm), intense research efforts have been placed on the development of NIR probes. In fact, NIR techniques have been designed to diagnose diseases, monitor the response to therapeutic treatment, track tumor development, and metastases, as well as evaluate the rehabilitation. NIR optical imaging has also been applied on pharmaceutical research, such as monitoring the biodistribution of drugs, and visualizing the targeted delivery of drug carriers in living animal subjects. In addition to imaging applications, QDs have potential in cancer photodynamic therapy as photosensitizers, since ultraviolet (UV) light and laser irradiation can induce them to generate reactive oxygen species (ROS) such as $\cdot\text{OH}$ and $\cdot\text{O}_2^-$, cytotoxic single oxygen ($^1\text{O}_2$) and toxic heavy metal ions. Majority of the work on QD biomedical applications as of yet has been focused on fluorescence imaging and very little has been exploited the efficacy of QDs for cancer therapy.

In our study, we report on a unique property of the targeted NIR QDs with brown or close to black colors in bright fields namely that they can rapidly convert light energy into heat after laser irradiation. This property is potentially useful for cancer photothermal therapy and was exploited for imaging cancer cells.



Schematic representation of (a) Photothermal ablation of NIR QDs on cancer cells, (b) Principle of deep tissue imaging.

N°O3a-4

**SAFETY OF NANOVECTORS: CYTOTOXICITY ASSESSMENT OF NEW SELF-EMULSIFYING
MULTIPLE W/O/W NANOEMULSIONS**

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Three W/O/W multiple nanoemulsions have been designed with the aim to be used as vector for drug delivery for both polar and non polar sensible drugs and for parenteral and oral routes. The spontaneous emulsification process is of great interest for including drugs sensible to high shear stress and temperature. The main drawback of self-emulsifying nanoemulsions is the need to include high quantities of surfactants which might induce cell toxicity. The aim of the work was to assess the cytotoxicity at the earlier stage of the development of 3 formulations previously found to exhibit good design characteristics. Nanoemulsions are blend of oily phase of medium chain triglyceride, surfactant mixture (7:3) (a) Polysorbate 85/Labrasol[®] (b) Polysorbate 85/Cremophor[®] EL (c) Glycerol/Polysorbate 85 and water. Cell viability was assessed with Alamar Blue (AB) test and apoptotic mechanism by chromatin condensation (Hoechst 33342 test) and P2X7 cell death receptor activation (Yo-Pro-1 test). Two of the three formulations, a & b exhibited cytotoxicity assessed by the AB test on epithelial cells at t=24h, IC₅₀=10.2 mg/ml and 11.8 mg/ml for a and b formulations respectively. According to our results and published data, Labrasol[®] and Cremophor[®] EL were suspected to be responsible of the cytotoxic effect of the formulations. IC₅₀ expressed in surfactant gave 0.51 mg/ml and 0.56 mg/ml for Labrasol[®] and Cremophor[®] EL respectively. In both cases, the cytotoxicity was due to an apoptotic mechanism evidenced by chromatin condensation and P2X7 cell death receptor activation (fig.1). On the contrary, no cytotoxicity was found with the formulation c and neither chromatin condensation nor P2X7 activation was found between 10 and 30 mg/ml concentration of the emulsion. Involvement of P2X7 cell death receptor activation would limit the development of the a & b formulations for chronic administration even for oral route. In opposite, the c formulation including glycerol and Polysorbate 85 will be of great interest for both IV and oral routes. Our work showed that P2X7 cell death receptor activation appeared as a good biomarker for apoptosis and nanovector toxic effect assessment. Moreover, it showed the interest of early toxicity studies of the nanovectors before considering further developments as drug safety delivery systems.

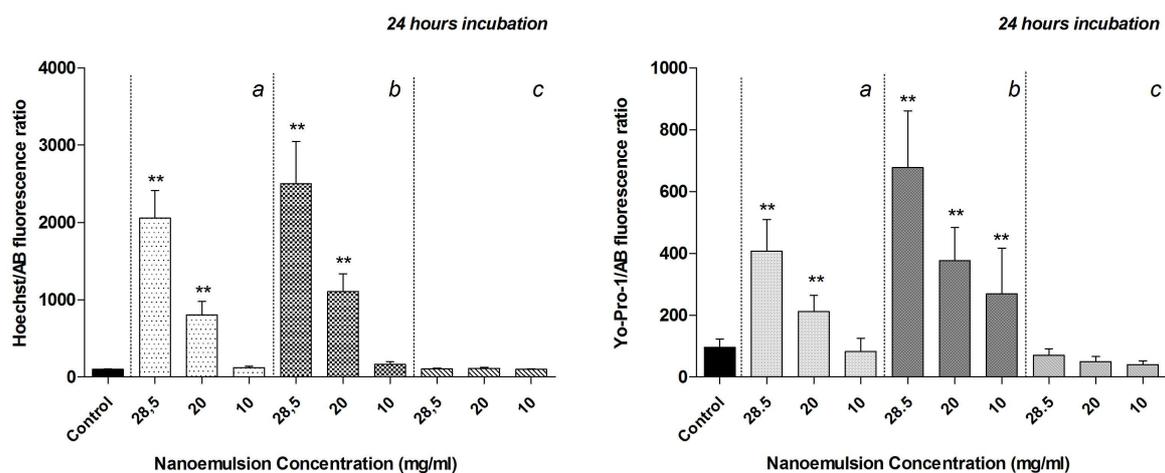


Figure1: Apoptotic assessment- Left graph: chromatin condensation (Hoechst 33342) Right graph: P2X7 cell death receptor activation (Yo-Pro-1 test) after 24 h incubation with the a, b, c multiple W/O/W nanoemulsions formulations. Ratio significance (**) p<0.001 compared to the control cells without nanoemulsion.

N°O3a-5

SURFACE LIGAND DEPENDENT FATE AND TOXICITY OF ZNO NP IN HepG2 CELLS

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The use of cell based systems to test the toxicity of nanoparticles (NP) involves placing the materials in biological media. This leads to interaction of the NP with the surrounding matrix and can lead to ligand exchange at the NP surface. These reactions are strongly depend on the chemical composition, size, shape, net charge and most importantly, on the initial surface functionality of NP^{1,2}, with structural and functional changes determining their physicochemical properties and bioactivity. Therefore, it is important to characterise NP in biologically relevant media to measure these changes and to understand their impact on toxicity measurements³.

In this study we examined how ZnO NP, plain or capped with different ligands, change when in contact with biological media and how these changes impact on both cellular uptake and their toxic effect. We show that over a 24h period ZnO NP capped with weakly bound large proteins undergo substantial changes in their size and net charge distribution, due to the surface ligand exchange. The kinetic of the observed process depends on the reaction environment and increases with temperature. Conversely, NP capped with a tight monolayer of small organic molecules or with covalently conjugated proteins are less prone to the ligand exchange and show good stability in serum containing media over the test period. Structural changes in the inorganic core (i.e. dissolution rate) during the exchange reactions, as well as the type and extent of NP surface coverage with the initial and exchanged ligands also depend on the initial surface functionality and are more significant for the NP capped with loosely bound ligands. Physicochemical properties of ZnO NP also strongly influence their impact on cell behaviour, including the entry route into cells, the uptake rate and the levels of toxicity, with NP with strongly bound ligands having the lowest impact.

In summary, NP undergo rapid and dramatic changes in their physicochemical properties in biological media, which in turn impacts their effect on cells. Therefore, it is important to characterise these changes in order to understand the mechanisms which underlay the toxicity of NP and how they are linked with the NP characteristics.

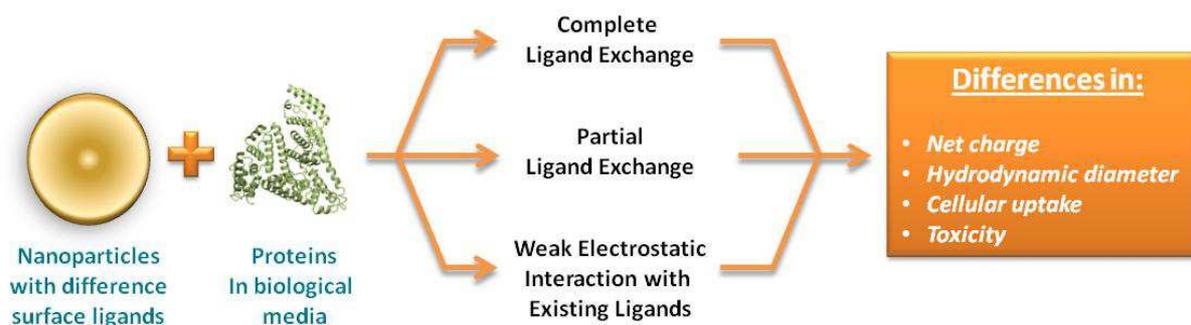


Figure 1. Schematic illustration of NP behaviour in contact with serum proteins and the main consequences of ligand exchange.

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N°O3a-6

ECSIN'S METHODOLOGICAL APPROACH FOR HAZARD EVALUATION OF ENGINEERED NANOMATERIALS

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The increasing production volumes and commercialization of engineered nanomaterials (ENM), together with data on their higher biological reactivity when compared to bulk counterpart and ability to cross biological barriers, have caused concerns about their potential impacts on the health and safety of both humans and the environment. A multidisciplinary component of the scientific community has been called to evaluate the real risks associated with the use of products containing ENM, and is today in the process of developing specific definitions and testing strategies for nanomaterials.

At ECSIN we are developing an integrated multidisciplinary methodological approach for the evaluation of the biological effects of ENM on the environment and human health. While our testing strategy agrees with the most widely advanced line of work at the European level, the choice of methods and optimization of protocols is made with an extended treatment of details. Our attention to the methodological and technical details is based on the acknowledgment that the innovative characteristics of matter at the nano-size range may influence the existing testing methods in a partially unpredictable manner, an aspect which is frequently recognized at the discussion level but oftentimes disregarded at the laboratory bench level.

This work outlines the most important steps of our testing approach. In particular, each step will be briefly discussed in terms of potential technical and methodological pitfalls that we have encountered, and which are often ignored in nanotoxicology research. The final aim is to draw attention to the need of preliminary studies in developing reliable tests, a crucial aspect to confirm the suitability of the chosen analytical and toxicological methods to be used for the specific tested nanoparticle, and to express the idea that in nanotoxicology, "devil is in the detail".

N°O3a-7

DEGRADED NANOCOMPOSITES: A COMBINED PHYSICO-CHEMICAL AND TOXICOGENOMICS APPROACH IN CACO-2 CELLS.

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Whereas literature about toxicity of nanoparticles (NPs) is growing up, very few is currently available on nanocomposites allying NPs and other components (protective layers, polymers), and still less about the fate of nanocomposites during their life-cycle. These nanocomposites spread in the environment and might release NPs as degradation residues. Indeed, degradation by the environment (exposure to UV, water contact) of protective layers could profoundly alter the physicochemical properties of NCs and modify their toxicity.

This work focuses on nanocomposites of titanium and cerium dioxide, and their potential interaction with the intestinal barrier modelized by Caco-2 cells. To simulate environmental exposure, suspensions of nanocomposites in water were degraded under UV-light. A possible degradation of nanocomposites after ingestion was performed using a simulated gastric medium. Nanocomposites sizes and crystal structure were characterized by DLS and TEM. Multiparametric viability tests (ATP, XTT, Trypan Blue) were performed on Caco-2 cells exposed to nanocomposites with no toxic effect up to 100 µg/mL over 72h. The exposed cells were imaged with SEM and nanocomposites internalization was researched by TEM. The cell morphology remained intact, attested by SEM, and internalization was not detected by TEM. Gene expression microarray analyses were performed to look for changes in cellular functions. For TiO₂ NCs, gene expression analyses using oligomicroarrays did not show any change versus unexposed cells at 10 µg/mL. TiO₂, degraded or not, do not seem very harmful to Caco-2 cells at environmental concentrations.

For CeO₂ nanocomposites, UV and acid degraded, adsorption is clearly seen on the cell surface by TEM, but internalization was not detected. The gene expression analyses reinforce these results by highlighting a default in the synthesis of small biomolecules, likely due to the coating of CeO₂ nanocomposites on cell membranes, preventing the assimilation of nutrients, thus inducing cell suffering.

N°O3a-8

**PRISTINE GRAPHENE ACTIVATES MACROPHAGES TO PRODUCE CYTOKINES/CHEMOKINES
VIA TLR- AND NF-KAPPA B-RELATED SIGNALING PATHWAYS**

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Graphene may have attractive properties for some biomedical applications, but its potential adverse biological effects, in particular, possible modulation of immune responses, require further investigation. Macrophages are one of the most important effector cells of the innate immune system, and play pivotal roles in the response to graphene exposure. However, little is known about the influence of exposure to low concentrations of graphene on the function of macrophages. In the present investigation, we demonstrate the biological effects of sub-cytotoxic concentrations of commercial pristine graphene on both primary murine macrophages and immortalized macrophages. Graphene significantly stimulates the secretion of Th1/Th2 cytokines including IL-1alpha, IL-6, IL-10, TNF-alpha and GM-CSF as well as chemokines such as MCP-1, MIP-1alpha, MIP-1beta and RANTES, probably by activating TLR-mediated and NF-kappa B-dependent transcription. Furthermore, these graphene-induced factors alter the morphology of naïve macrophages by remodelling their actin assembly, decreasing their ability to adhere to the extracellular matrix, and attenuating their phagocytosis. This negative feedback of the immune response of macrophages by graphene-induced factors may play an important role in the prevention of their over-activation after graphene exposure. When macrophages were exposed to higher concentrations of graphene, cell death was initiated, probably via MAPK- and TGF-related pathways. These findings shed light on the interaction of graphene and macrophages in vitro. Further research is needed to systematically assess the biological responses of graphene, both to improve its safety and to contribute to the design of new biological applications.

N°O3b-1

**ASSESSMENT OF DIFFERENT METHODS STUDYING THE IMPACT OF CARBON
NANOMATERIALS ON PLATELET FUNCTION.**

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* Both authors have equally participated to the study reported here.

Objectives: We aimed to validate an universal, fast, accurate, reliable and relevant toxicological preclinical screening test to measure the potential impact on primary haemostasis of nanoparticles (NP) whatever their physicochemical properties. **Materials and methods:** Four types of carbon nanoparticles (Carbon Black (CB), Fullerenes (C60), Single Wall Carbon Nanotubes (SWCNT), Multi Wall Carbon Nanotubes (MWCNT)) considered as promising in medical applications were investigated. The interference of these nanoparticles on Light Transmission Aggregometry (LTA), Whole-blood Impedance Aggregometry (Multiplate[®]), Platelet Function Analyzer-100 (PFA-100[®]) and Impact-R[®] was studied before the assessment of their effect on platelet function (adhesion, activation and aggregation). Interference and functional impact were also analyzed by transmission- and scanning electron microscopy. **Results:** Maximal concentrations of C60, CB, SWCNT, MWCNT that may be tested with optical methods like LTA are 500, 10, 500 and 100 µg/ml, respectively. Each nanoparticle interferes by flux obstruction with PFA-100[®] at concentration higher than 10 µg/ml. Whole-blood impedance aggregometry was not considered as a suitable method because of the interaction between negatively charged nanoparticles and the impedance measures. Impact-R[®] showed absence of interference of C60, CB, SWCNT, MWCNT up to 250, 500, 500 and 250 µg/ml, respectively. Furthermore, the addition of Bovine Serum Albumin 7,4g/l (final concentration) to mimic human blood viscosity abolishes the interference of C60 and MWCNT. Below cut-offs without any interference, none of the nanoparticle has a significant effect on the platelet function, whatever the method used. **Conclusion:** Impact-R[®] is the most adapted test to assess the effect of manufactured NPs on platelet function.

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N°O3b-2

NITRIC OXIDE RELEASING-IRON OXIDE MAGNETIC NANOPARTICLES FOR BIOMEDICAL APPLICATIONS: CELL VIABILITY, APOPTOSIS AND CELL DEATH EVALUATIONS

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Nitric oxide (NO) is involved in several physiological and pathophysiological processes, such as the control of vascular tone, the inhibition of platelet aggregation, and the immune response against microbes. Thus, there is a great interest in the development of NO-releasing vehicles that are able to stabilize and release NO locally direct to the target site, in diverse biomedical applications. Superparamagnetic iron oxide nanoparticles (SPIONs) have been the topic of intensive investigations due to their important biomedical applications, including drug delivery. In this work, magnetic iron oxide nanoparticles were coated with thiol-containing hydrophilic ligands, mercaptosuccinic acid (MSA) and dimercaptosuccinic acid (DMSA). The free thiols (MSA and DMSA) on the surface of the coated nanoparticles were nitrosated, by adding sodium nitrite, leading to the formation of NO-releasing iron oxide nanoparticles. The cytotoxicity of this new NO-releasing nanomaterial was evaluated in cell line 3T3 and in human lymphocytes (Figure 1).

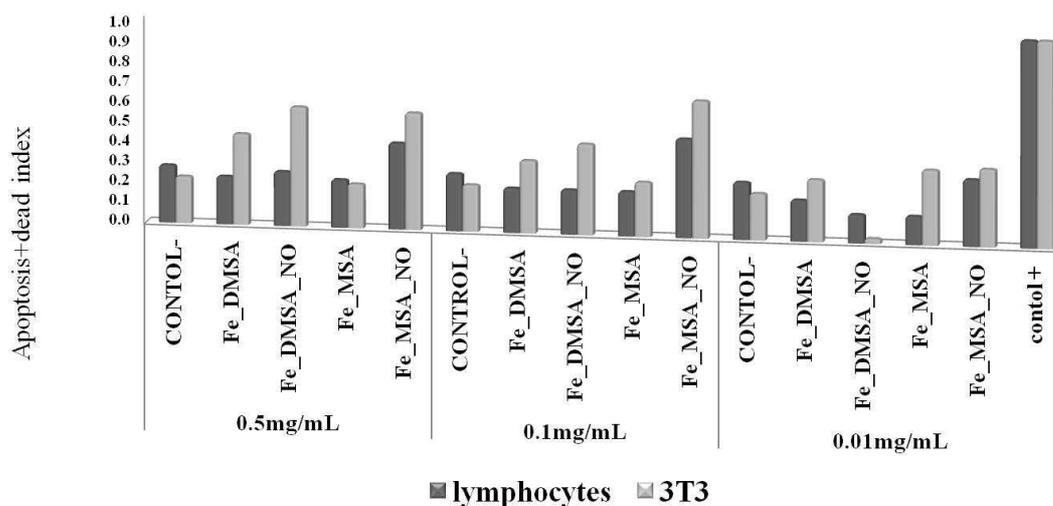


Figure 1. Apoptosis and cell death index for human lymphocytes and 3T3 cells treated with: iron oxide nanoparticles coated with: DMSA (Fe-DMSA), NO-coated DMSA (Fe-DMSA-NO), MSA (Fe-MSA), NO-coated MSA (Fe-MSA-NO). Concentrations: 0.5, 0.1 and 0.01 mg/mL. Incubation time: 1 h.

Initial results show that magnetic iron oxide nanoparticles coated with DMSA and MSA have low cytotoxicity effects for both cell types, and nanoparticles coated with MSA were found to be less cytotoxic, compared to nanoparticles coated with DMSA. Moreover, NO-releasing nanoparticles were found to increase apoptosis and cell death compared to the nanoparticles without NO. This effect was more evident for nanoparticles concentrations 0.5 and 0.1 mg/L and less evident for 0.01 mg/mL and the lymphocytes were less susceptible to this treatment, in comparison with 3T3 cell. These results show that the cytotoxic effects observed in cell culture can be attributed to the presence of NO on nanoparticle surface. Therefore, NO-releasing nanoparticles may result in important biomedical applications, such as the treatment of tumors, in which the nanoparticles can be guided to the target site through the application of a magnetic field, and release NO direct to the desired tissue/cell, where NO can have its cytotoxic effects.

N°O3b-3

TRANSLOCATION OF SiO₂ NANOPARTICLES ACROSS HUMAN BRONCHIAL EPITHELIAL CELLS

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Safe development and application of nanotechnologies in many fields will require more knowledge about their potential effects on human health; especially their ability to cross physiological barriers. Inhalation is a frequent mode of exposure to nanoparticles (NPs) and there is evidence of their ability to cross epithelial barriers to reach secondary organs via the bloodstream. Our aim is to study the translocation of three different sized NPs (16, 50, and 100 nm) across human bronchial epithelial cells. For this purpose we used the Calu-3 cell line which when it grows on a porous membrane, forms an efficient bronchial barrier. Indeed, by evaluating the transepithelial electrical resistance (TEER), as well as monitoring the paracellular transport of a fluorescent marker (Lucifer Yellow), we have shown that Calu-3 cells are able to form a tight epithelial monolayer after 14 days in culture when they were seeded on 3 µm-polycarbonate Transwell membranes.

To quantify translocation we used fluorescently labelled SiO₂ NPs (SiO₂-FITC NPs) and investigated their ability to cross the porous membrane in the absence of cells. Whatever the NP size, 24 h after applying NPs to the apical chamber, the NPs were recovered from the basal chamber with an increasing NPs concentration (up to 10%) when the applied apical concentrations were decreasing. The majority of the NPs remain trapped in the membrane. Furthermore upon addition of foetal calf serum (FCS) or DPL (DiPalmitoyl Lecithin), the transport of NPs increases by 5 or 30 times respectively. Confocal microscopy experiments were also carried out to observe the distribution of NPs in the membrane.

SiO₂-FITC NPs were non cytotoxic at concentrations up to 10µg/cm² and had no pro-inflammatory effect after 24 h of treatment as shown by studying cytokine release. However they dose-dependently induced the expression of MUC5AC a specific bronchial mucin. The translocation of NPs was size-dependent: 6% of the NPs applied to the apical chamber were recovered from the basal chamber when cells were exposed to the 16nm sized NPs whereas this percentage fell to 2% for the largest size. Confocal microscopy experiments were used to observe NPs internalization.

Experiments were also performed on Normal Human Bronchial Epithelial cells (NHBE) which are a more relevant model. Quantitative translocation assays cannot be done as these cells must be grown on low porosity membranes (0.4µm). Only morphological studies by confocal microscopy were performed on NHBE which exhibit normal (mucociliary) or pathological (squamous and mucosal) differentiation.

In conclusion, SiO₂-FITC NPs can cross the bronchial epithelial barrier at non cytotoxic concentrations, without inducing inflammation and affecting epithelial integrity. The mechanisms involved in this translocation are now under investigation.

This work was supported by grants from Anses project N° PNR-EST-2010/2/79 (TiSiTrans) and National Grant "Nanotrans".

N°O3b-4

DEVELOPMENT OF A DOSE-CONTROLLED MULTICULTURE CELL EXPOSURE CHAMBER FOR EFFICIENT DELIVERY OF AIRBORNE AND ENGINEERED NANOPARTICLES

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In order to study the various health influencing parameters related to engineered nanoparticles as well as to soot emitted by Diesel engine exhaust, there is an urgent need for appropriate sampling devices and methods for cell exposure studies (simulating the respiratory system) and associated biological and toxicological tests. The objective of the present work is the further advancement of an upgraded Multiculture Exposure Chamber (MEC) (Fig. 1) into a dose-controlled system for efficient delivery of nanoparticles to cells and its validation with various types of nanoparticles (diesel engine soot aggregates, engineered nanoparticles for various applications) with state-of-the-art nanoparticle measurement instrumentation to assess the local deposition of nanoparticles on the cell cultures. The dose of nanoparticles to which cell cultures are being exposed is evaluated in the normal operation of the in vitro cell culture exposure chamber based on measurements of the size specific nanoparticle collection efficiency of a free of cells device. Measurements so far have demonstrated that the average efficiency in delivering nanoparticles in the MEC is approximately 80%. The nanoparticle deposition is being controlled and quantified by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) (Fig. 2). Analysis and design of the MEC employs Computational Fluid Dynamics (CFD) and true to geometry representations of nanoparticles with the aim to assess the uniformity of nanoparticle deposition among the culture medium wells. Final validation of the dose-controlled cell exposure system will be performed with exposure tests of real cell cultures to various types of nanoparticles. The efficient delivery of engineered nanoparticles will be demonstrated by the measurable toxicity induced to the cells by the exposure to nanoparticles. The toxicity is being monitored by the Reactive Oxygen Species (ROS) generation potential of nanoparticle suspensions (e.g. free radicals and peroxides generation), thus expressing the oxidative stress of the cells which can cause extensive cellular damage or damage on DNA.

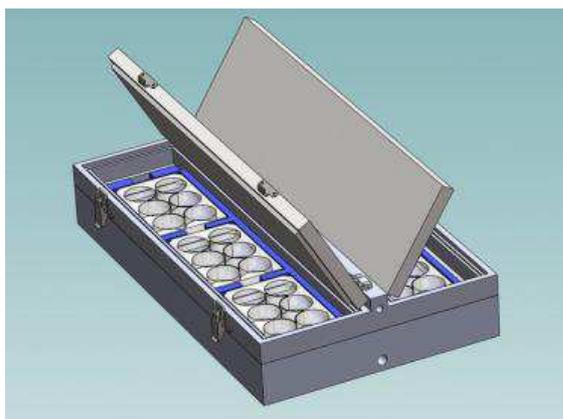


Figure 1: The upgraded Multiculture Exposure Chamber (MEC)

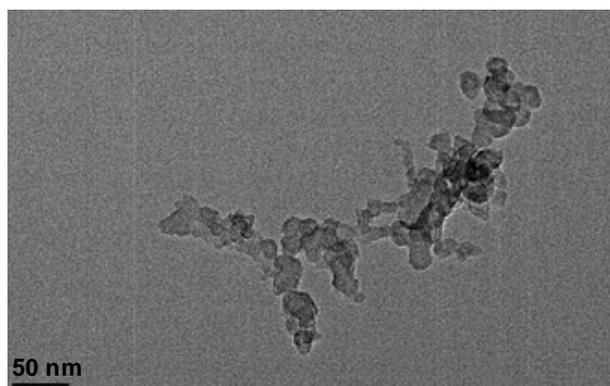


Figure 2: Representative TEM picture of soot nanoparticles deposited in the MEC

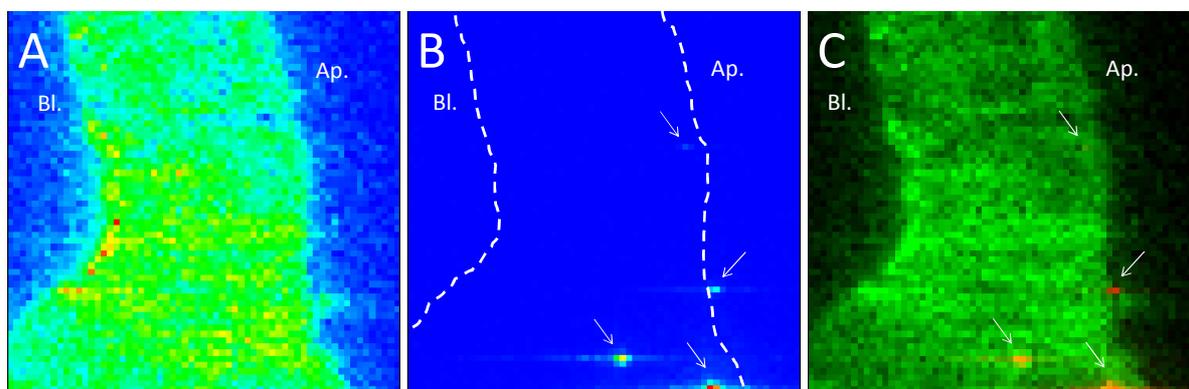
N°O3b-5

IN VITRO, EX VIVO AND IN VIVO TRANSLOCATION OF TITANIUM DIOXIDE NANOPARTICLES THROUGH THE GASTROINTESTINAL BARRIER, TOXICOLOGICAL CONSEQUENCES

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Nanoparticles (NPs) are presently introduced in a growing number of commercial products, particularly in the food industry as food additives, packaging or as dietary supplements. Among them, titanium dioxide is used for whitening and brightening foods. It is also used in daily use hygiene products such as toothpaste or sunscreens, or orally-administered drugs. Effects of TiO₂-NPs on gastrointestinal (GI) tract, as well as their translocation through the GI barrier, are poorly investigated.

We focused on the translocation of TiO₂ NPs through different models of GI epithelium: simple cell models and co-cultures providing properties of a GI barrier to the *in vitro* culture. These models were exposed to well characterized anatase and rutile NPs for 6h-48h, on the apical pole of the monolayer. TiO₂ NP cellular uptake and translocation was followed by transmission electronic microscopy and μ -XRF elemental mapping, coupled to XAS analysis of Ti atoms local structure. Our conclusions were then validated in an *ex vivo* model and *in vivo* on mice orally exposed to TiO₂ NPs. Finally TiO₂ NP fate was evaluated when submitted to a *in vitro* digestion model, consisting in incubating successively anatase or rutile NPs in various fluids mimicking the different compartments of a GI tract. The originality of this project relies on the panel of microscopy techniques implemented to investigate digestive barrier translocation, bringing together biologists, chemists and physicists in a pluridisciplinary research program.



Distribution of Os (A), as an image of cell contour, and of Ti (B), as an image of TiO₂-NP distribution. C is a superimposition of both Os and Ti images. μ -X-ray fluorescence mapping was performed on Caco-2 cells exposed for 48 h to rutile, 20 nm NPs.

Reference:

G. Veronesi, E. Brun, B. Fayard, M. Cotte, M. Carrière. Structural properties of rutile TiO₂ nanoparticles accumulated in a model of gastrointestinal epithelium elucidated by micro-beam X-ray Absorption Fine Structure spectroscopy. *Applied Physics Letters*, 100(21)

N°O3b-6

VARIATION IN SILVER NANOPARTICLES TOXICITY IN THE PRESENCE OF PHENOLIC COMPOUNDS

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Interaction of nanoparticles (NPs) with food components could alter the uptake, bioaccumulation and biotransformation of NPs, as well as their potential toxic effects. To date, little is known about the uptake and toxicokinetic processes following oral exposure to NPs, particularly in relation to their ingestion with food matrices. Phenolic compounds (PCs) represent an important part of human micronutrients and widely occur in fruits, vegetables, wine, tea, chocolate, etc. This study aimed at detecting the possible influence of some PCs, *i.e.* quercetin – Q (flavonol) and resveratrol – R (stilbene), on silver NPs toxicity *in vitro*.

The influence of Ag-NPs (< 20 nm, 10 – 90 µg/ml, Mercator GmbH, DE) was studied on Caco-2 cells (passage 10 – 30, clone N1, received from Dr. M. Rescigno, University of Milan-Bicocca, IT). PCs were applied in a range of 10 – 150 µM, representing realistic average intestinal concentrations upon food ingestion. The cytotoxic effect of NPs was assessed by 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich, Steinheim, UK). The influence of Ag-NPs on cell monolayer integrity was evaluated by transepithelial electrical resistance (TEER), as well as by Lucifer Yellow – LY (Sigma-Aldrich, Steinheim, UK) passage, conducted on 21 days cultures in Transwell Permeable Supports of 12 mm (Corning Inc., NY).

It was revealed that silver NPs possess cytotoxic effect on Caco-2 cells with an EC₅₀ of ca. 40 µg/ml. To study the effect of Q and R, the concentrations below 50 and 100 µM were chosen, respectively, as higher concentrations of these PCs were toxic for proliferating cells. A protective effect of 50 µM Q was observed on Caco-2 cells upon incubation with Ag-NPs at 45 µg/ml, where the cell viability was increased up to about 60% and reached to the control level. The passage of the LY – a paracellular passage marker, confirmed the received results, where the Q lowered the Ag-NPs-induced elevated dye passage until the control level. In contrast, R possessed a synergistic effect and decreased the cell viability up to 25% compared with that of Ag-NPs alone. The above-mentioned protector and synergistic effects were dependent on Q and R concentrations, respectively. These data were in correlation with TEER measurements, where Q recovered the TEER value until the control level, whereas R increased the effect of Ag-NPs, leading to an additional decrease of TEER value.

Received data suggest that under the influence of Ag-NPs, the permeability of Caco-2 cell monolayer increases, which could be connected with the reorganisation of the tight junction proteins – the gatekeepers of the paracellular passage. It was demonstrated that Q and R alter the effect caused by Ag-NPs on intestinal epithelial cells and, moreover, had opposite effects. Further studies are necessary to reveal the primary targets of Ag-NPs, as well as mechanisms of joint action of NPs with PCs, whether protective or not.

* This work was supported by a grant of the Belgian Federal Science Policy (BELSPO).

N°O3b-7

DEVELOPMENT OF A PBPK MODEL FOR IONIC AND NANOPARTICULATE SILVER

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According to the Woodrow Wilson Database on Emerging Nanotechnologies [1], nano-silver is by far the most common nanomaterial used in consumer products. Moreover, owing to its broad-spectrum antibiotic properties nano-silver is used in a wide range of different applications. Potential exposure routes include dermal uptake (e.g. wound dressings, clothes), inhalation (e.g. sprays, occupationally) and ingestion (e.g. food boxes, dietary supplements). Hence, under unfavourable conditions a large total consumer exposure to nano-silver is possible.

Our project aims at comparing these different exposure routes and also ionic silver to nanoparticulate silver exposure. Therefore, a physiologically based pharmacokinetic (PBPK) model was developed, which is designed as multi-compartment model. The model calculates the silver mass flow within the human body, including (nano-) silver absorption via different exposure routes, and subsequent distribution, metabolism and excretion (see Figure 1). Therefore, for ionic and nanoparticulate silver two separate modelling approaches were used. The ionic silver model was based on the mercapto pathway and, hence, closely linked to the distribution of glutathione in the body. For the nano-silver model the organs were grouped according to their capillary type, and to whether or not they are part of the mononuclear phagocyte system (MPS). Distribution, metabolism and excretion parameters were calibrated using in-vivo data of organ and tissue silver levels of rats and were then extrapolated to humans.

All exposure routes were extensively validated by comparing the PBPK model to in-vivo and biomonitoring data from the literature. Furthermore, we used our PBPK model to evaluate and compare organ and tissue silver levels in the human body, caused by various (nano-) silver containing consumer products. In all exposure scenarios (nano-) silver levels were at least a factor of 100 below reported no-observed-effect-levels (based on in-vitro studies), although very conservative application assumptions were made. Potentially critical levels were only exceeded in one scenario, where exposure data from a biomonitoring study of occupationally exposed workers was used (faecal and serum silver levels were used for validation).

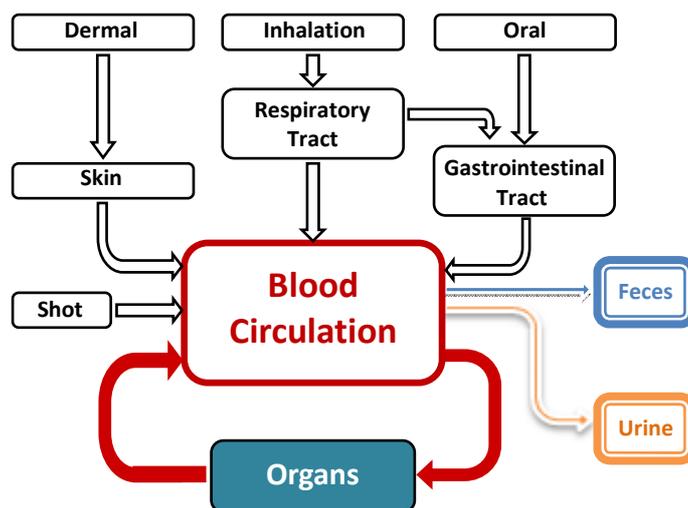


Figure 1: Simplified compartment flow chart of the PBPK

The Project is funded by the Federal Office of Public Health (FOPH), Switzerland.

[1] Woodrow Wilson International Center for Scholars. 2012. Consumer Product Analysis. http://www.nanotechproject.org/inventories/consumer/analysis_draft. Accessed: 8/07/2012

N°O3b-8

PREDICTIVE TESTS TO EVALUATE OXIDATIVE POTENTIAL OF ENGINEERED NANOMATERIALS

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Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and antioxidant defences able to protect from the resulting damage. Oxidative stress constitutes one of the principal injury mechanisms through which particulate toxicants (asbestos, crystalline silica, hard metals) and engineered nanomaterials can induce adverse health effects. ROS may be generated indirectly by activated cells and/or directly at the surface of the material. The occurrence of these processes depends upon the type of material. Many authors have recently demonstrated that metal oxides and carbon-based nanoparticles are influence (increasing or decreasing) the generation of oxygen radicals in a cell environment¹. Metal oxide, such as iron oxides, crystalline silica, and titanium dioxide are able to generate free radicals via different mechanisms causing an imbalance within oxidant species. The increase of ROS species may lead to inflammatory responses and in some cases to the development of cancer. On the other hand carbon-based nanomaterials, such as fullerene, carbon nanotubes, carbon black as well as cerium dioxide are able to scavenge the free radicals generated acting as antioxidant. This property may be exploited in medicine to mitigate oxidative stress associated to inflammatory and degenerative diseases².

The high number of new engineered nanomaterials which are introduced in the market are exponentially increasing. Therefore the definition of toxicological strategies are urgently needed. The development of acellular screening tests will make possible the reduction of the number of in vitro and in vivo tests to be performed. An integrated protocol that may be used to predict the oxidant/antioxidant potential of engineered nanoparticles will be here presented. The oxidative potential of the powders is evaluated by measuring the capability to generate or to scavenge free radical species by means of EPR spectroscopy/spin-trapping or probing technique. The abilities to cause oxidative damage to lipids, proteins or DNA were evaluated by means to UV/Vis spectrophotometry, SDS-page and DNA agarose gel electrophoresis respectively.

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N°O3b-9

COMPARISON OF TOXICITY OF UNCOATED AND COATED SILVER NANOPARTICLES

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Due to their antimicrobial properties, silver nanoparticles (Ag-NPs) have been widely used in consumer products. Despite the large number of consumer products containing Ag-NPs produced each year, little is known about the human health risks and environmental effects of these NPs. In the present study, we investigate the toxicity of uncoated colloidal (20 nm, 40 nm, 60 nm and 80 nm) and OECD sponsored citrate and PVP coated (10 nm, 50 nm and 75 nm) Ag-NPs in J774A.1 macrophage and HT29 epithelial cells. Test cells were exposed to different concentrations (silver content) of Ag-NPs for 24 h. The study employed the MTT assay to assess cell viability (ie. cytotoxicity). Confocal and transmission electron microscopy were used to examine sub-cellular structures and reactive oxygen species (ROS) production. Multiplex bead-based assays were performed to measure cytokine levels and enzymatic assays were used to detect glutathione (GSH) levels and superoxide dismutase (SOD) activity. At a concentration of 1 µg/ml, uncoated Ag-NPs caused 20-40% drop in cell viability, and further analysis showed 20 nm and 40 nm particles caused 10% more cytotoxicity compared 60 nm and 80 nm particles, indicating size-dependent effects. Exposures of cells to coated AgNPs resulted in drops in cell viability starting at 25 µg/ml. The effects of coated Ag-NPs on cell viability also appeared to be size-dependent. PVP coated particles induced greater cytotoxicity than citrate coated particles. Changes in sub-cellular architecture were observed in J774A.1 cells from exposures to uncoated 20 nm and 40 nm particles at 1 µg/ml and from exposures to both citrate and PVP coated 10 nm and 50 nm particles at 50 µg/ml. At 1 µg/ml, uncoated Ag-NPs caused a decrease in the expression of selected cytokines including TNF-α in J774A.1 and IL-8 in HT29 cells, compared to the controls. By contrast, both citrate and PVP coated Ag-NPs increased the expression of these cytokines at higher concentrations (25 and 50 µg/ml), and PVP coated particles caused greater elevation of cytokines compared to citrate coated particles. While uncoated Ag-NPs resulted in depletion of GSH and increased SOD in test cells in a size-dependent manner at 1 µg/ml, coated Ag-NPs caused insignificant changes in GSH and SOD, even at the highest test concentrations. Uncoated (20nm and 40nm) at 1 ug/ml and coated Ag-NPs (10nm-PVP) at 50 ug/ml induced slight increases in ROS production, compared to the control. Our data showed that uncoated Ag-NPs are more toxic than coated Ag-NPs. While uncoated NPs appeared to suppress inflammatory responses and caused oxidative stress in test cells, coated Ag-NPs seem to induce toxic effects through up-regulation of cytokines. Our findings suggest that the toxicity of Ag-NPs is size- and coating-dependent. The study provides scientific data that helps to enhance the insight on the health impact of Ag-NPs.

N° O3b-10

NANO-TITANIUM DIOXIDE MODULATES THE DERMAL SENSITIZATION POTENCY OF DNCB

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We determined the ability of a model nanoparticle (NP) (titanium dioxide, TiO₂) to modulate sensitization induced by a known potent dermal sensitizer (dinitrochlorobenzene) using a variant of the local lymph node assay called lymph node proliferation assay.

BALB/c mice received sub-cutaneous injections of vehicle (2.5mM sodium citrate), TiO₂ NPs (0.004, 0.04 or 0.4 mg/ml) or pigment particles (0.04 mg/ml) both stabilized in sodium citrate buffer at the base of each ear (2x50µl), before receiving dermal applications (on both ears) of 2,4-Dinitrochlorobenzene (DNCB) (2x25µl of 0.1%) or its vehicle (acetone olive oil – AOO (4:1)) on days 0, 1 and 2. On day 5, the stimulation index (SI) was calculated as a ratio of ³HTdR incorporation in lymphocytes from DNBC-treated mice and AOO-treated controls. In a second experiment the EC₃-value for DNCB (0 to 0.1%) was assessed in the absence or presence of 0.04 mg/ml TiO₂. In a third experiment, the lymphocyte subpopulations and the cytokine secretion profile were analyzed after TiO₂ (0.04 mg/ml) and DNCB (0.1%) treatment.

Injection of NPs in AOO-treated control mice did not have any effect on LN proliferation. DNCB sensitization resulted in LN proliferation, which was further increased by injection of TiO₂ NPs before DNCB sensitization. The EC₃ of DNCB, with prior injection of vehicle control was 0.041%, while injection with TiO₂ decreased the EC₃ of DNCB to 0.015%. TiO₂ NPs pre-treatment did not alter the lymphocyte subpopulations, but significantly increased the level of IL-4 and decreased IL-10 production in DNCB treated animals.

In conclusion, our study demonstrates that administration of nano-TiO₂ increases the dermal sensitization potency of DNCB, by augmenting a Th₂ response, showing the immunomodulatory abilities of NPs.

Sponsor

EU-FP7: NanoHouse

N°O3b-11

USING A PBPK MODEL TO STUDY THE INFLUENCE OF DIFFERENT CHARACTERISTICS OF NANOPARTICLES ON THEIR BIODISTRIBUTION

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The studies on potential health risks possessed by engineered nanoparticles (NPs) have been growing rapidly. However, detailed and systemic knowledge on the uptake and biodistribution of NPs in body is still limited. Moreover, there is a need to characterize the relation between the characteristics of NPs (size, surface modifications, etc.) and their behaviors in the body. The aim of this study is to explore how these characteristics will influence the NPs uptake and biodistribution. We have successfully developed a Physiologically Based Pharmacokinetic (PBPK) model for the biodistribution of polyethylene glycol-coated polyacrylamide NPs in rats, modeling the capture and removal of NPs by macrophages. Based on this PBPK model, the behaviors of other nanoparticles (polymeric, quantum dot, silver, titanium oxide and cerium oxide NPs) are investigated, based on data from several experiments published in the literature. Size is one of the important properties to consider. Our model parameterization suggests that the uptake rate by macrophages will decrease by up to 20-fold as the size of nanoparticles increases from 30 nm to 300 nm while the uptake capacity for mass increases up to 6-fold. This could indicate that the macrophages are saturated by the number of NPs rather than absolute mass. Nevertheless, surface modification, such as polyethylene glycol coating, reduces the uptake rate by macrophages. Besides, bio-elimination could have significant impact on the biodistribution of certain NPs. With macrophages serving as a deposit of NPs, these influences of different characteristics of NPs to the behavior of macrophages could affect the fate of NPs in the body not only during the initial uptake within the first hour but also in long-term at the kinetic and dynamic levels.

N°O3b-12-

CYTOTOXICITY OF SOLID LIPID NANOPARTICLE AND NANOSTRUCTURED LIPID CARRIERS WITH LOCAL ANESTHETIC DIBUCAINE FOR TOPICAL APPLICATION

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Dibucaine (DBC) is one of the most powerful long-lasting local anesthetics. However, it is also considered to be fairly toxic to the CNS, being its use limited to surface anesthesia. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) have attracted attention as promising carriers for drug delivery. The aim of this study was to develop and to evaluate the cytotoxic activity of DBC-loaded SLN and NLC against 3T3 fibroblast and HaCat keratinocytes cells. SLN and NLC contained myristyl myristate and myglyol as their lipid matrices, respectively, plus a surfactant (Pluronic F68). The SLN and NLC, prepared with the high pressure procedure were characterized in terms in their diameter, size distribution, surface charge and toxicity against cell cultures. The size of SLN (Fig. 1.a) and NLC (Fig. 1.b) were 234.33 ± 42.87 nm and 166.62 ± 14.96 nm, respectively; statistical analysis detected no significant differences ($p > 0.05$, Unpaired t-test) in size after DBC loading. The polydispersity index was kept below 0.25, which is within the range expected for homogeneous dispersed colloids. As for the Zeta potential, positive surface charges (-29 - -15 mV) were observed for all formulations. Free DBC decreased the viability of 3T3 and HaCat cells reaching the IC_{50} (concentration required for 50% cell viability) at 0.3 mM. Encapsulation of DBC in NLC and SLN carriers significantly decreased its intrinsic cytotoxic effect ($p > 0.001$, ANOVA and Turkey-Kramer test). SLN or NLC (without DBC) and DBC-loaded nanoparticles were not toxic up to 4.2 mM and 0.3 mM respectively, to 3T3 and HaCat cells. In conclusion, encapsulation of DBC in SLN and NLC decreased the *in vitro* toxicity of the local anesthetic, indicating the great potential of these lipid-based nanocarriers for clinical (dermal anesthesia) applications. *In vivo* studies are being carried in our laboratory to test the antinociceptive effects of both drug delivery systems.

Supported by FAPESP (# 06/00121-9), CAPES, INOMAT (MCT/CNPq) and Brazilian Network in Nanotoxicology (MCT/CNPq), Brazil

(a)(b)

Fig. 1: TEM image of solid lipid nanoparticles (a) and nanostructured lipid carriers (b) containing dibucaine (DBC).

N°O3c-1

**TISSUE DISTRIBUTION AND CLEARANCE OF TITANIUM DIOXIDE NANOPARTICLES AFTER
INTRAVENOUS ADMINISTRATION AND INTRATRACHEAL INSTILLATION**

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Titanium dioxide (TiO₂) is insoluble and inert and shows low toxicity in humans; therefore, it is used as an additive in food products. However, TiO₂ nanoparticles could be more toxic than pigment-grade TiO₂ particles because of the high reactivity and slow clearance owing to their size (Donaldson et al. 2001, Oberdörster et al. 2005). In this study, we aimed to determine the clearance and translocation of TiO₂ nanoparticles by using a highly sensitive method involving sample preparation, except dissection, under a clean environment and double-focusing sector field inductively coupled plasma-mass spectrometry (ICP-SFMS) for the analysis of TiO₂ without labeling. We selected Degussa P25 as the target TiO₂ particle because, in several studies, it has been reported to have stronger effects than those of other TiO₂ particles.

Initially, we determined the clearance of TiO₂ from the circulation in rats after intravenous administration of 1 mg/kg of TiO₂. Then, we determined the tissue distribution and decrease in the concentration of TiO₂ over time in the liver, kidney, spleen, lung, brain, and the other organs after intravenous administration of 1 mg/kg of TiO₂ and after intratracheal instillation of 2.3 mg/kg of TiO₂ in rats. In addition, we measured the concentration of TiO₂ in the feces and urine of rats after intravenous administration and intratracheal instillation. The detection limits were 2.5×10^{-7} mg/mL for blood and urine and 1.0×10^{-6} mg/g tissue for organ tissues; therefore, we were able to measure the distribution and clearance of TiO₂ in the organs in which these parameters could not be previously determined.

Our results showed that more than 90% of TiO₂ was present in the liver, and 2% and 0.2% was present in the spleen and lung, respectively, at 6 h after intravenous administration. The TiO₂ burden in the liver and spleen did not decrease until 30 days after its administration, while that in the lung, kidney, and blood decreased over time. To determine the clearance trend and the clearance parameter, the TiO₂ burden decays in the lung and kidney were estimated by fitting with a 2-step decay model and 1-step decay model, respectively. Three days after the intratracheal instillation, 7 mL of the bronchoalveolar lavage fluid (BALF) was sampled twice and the organs were dissected. Our results showed that more than 90% of TiO₂ was present in the lung, while the TiO₂ burdens in the right and left mediastinal lymph nodes and in the parathymic lymph node were 0.04%, 0.03%, and 0.2% of the administered dose, respectively. The concentrations of Ti in the feces and urine of rats who received intravenous TiO₂ were not different from the corresponding concentrations in the rats in the control group, because the Ti content in the feed was quite high (approximately, 3.0 µg/g). Ti concentration in the feces decreased over time until 3 days after intratracheal instillation and was higher in the BALF sampling group than in the BALF non-sampling group. This finding suggested that the deposited TiO₂ particles could be cleared and excreted via the bronchial pathway and esophagus in a few days after its instillation.

N°O3c-2

**A DYNAMIC CO-CULTURE MODEL RESEMBLING THE ALVEOLO-CAPILLARY BARRIER TO
STUDY TOXICITY AND TRANSLOCATION OF NANOPARTICLES**

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In order to study the toxic effects and translocation of nanoparticles (NPs) from the lung to the systemic circulation, we created a co-culture model resembling the alveolo-capillary barrier via cellular interactions between monocytes, epithelial and endothelial cells.

The human pulmonary epithelial 16HBE14o- cell line (16HBE) and human umbilical vein endothelial cells (HUVEC) were seeded on opposite sides of a permeable membrane support. Five days after seeding, the bi-culture reaches a maximum transepithelial electrical resistance (TEER) of $824.18 \pm 90.79 \Omega \cdot \text{cm}^2$, which is significantly higher compared to the 16HBE monolayer ($522.97 \pm 59.96 \Omega \cdot \text{cm}^2$), while the HUVEC monolayers do not produce TEER. The presence of tight junctions was confirmed by electron microscopy. On the fifth day in culture, monocytes (THP-1) or macrophages (PMA-activated THP-1 cells), in a ratio of 1 to 10 16HBE cells, or conditioned medium of monocytes or macrophages was added to bi-cultures. After 24 hours, TEER was significantly decreased in the tri-culture with macrophages (37.79 ± 2.53 % of baseline), but increased with conditioned medium of monocytes (152.94 ± 5.06 %) compared to the control (110.63 ± 1.79 %). Due to the toxicity induced by PMA-activated macrophages we checked whether monocytes can be activated (to macrophage-like cells) in the presence of NPs. Autofluorescence was dose-dependently increased by silica NPs to values equal or higher than macrophages. Moreover, the appearance of activated monocytes resembled that of macrophages under the light microscope.

The results indicate a tight epithelial barrier and the presence of endothelial cells in the model accounts for significantly higher TEER values compared to the 16HBE monolayer. The ability of monocyte activation by exogenous substances completes the model and makes it suitable to study the effects of NP (and other substances) on the alveolo-capillary barrier.

EU-FP7: ENPRA; FWO:F.OS47.08 and IWT (agentschap voor Innovatie door Wetenschap en Technologie) Ref 101061

N°O3c-3

**COMMUNICATING NANOTOXICOLOGY: THREE EVALUATIONS USING *IN VITRO*
CENTRAL NERVE MODELS**

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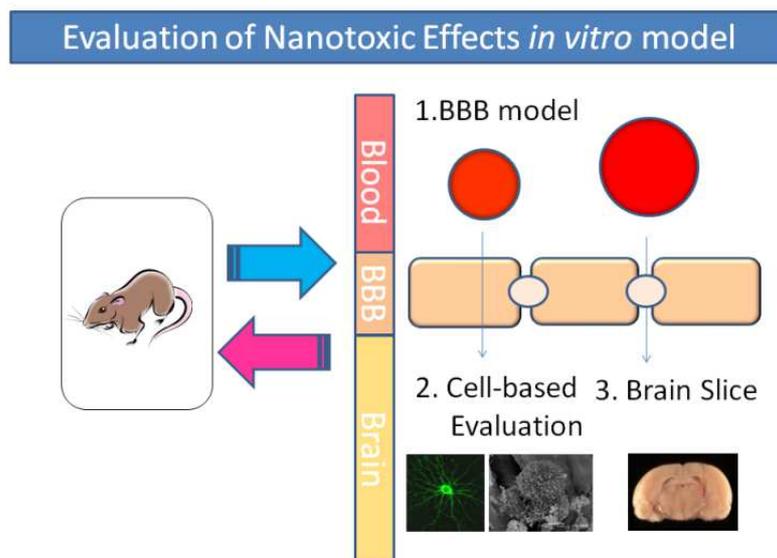
This study aims to develop easier assessment tools of nanoparticle's toxicological effects to the central nerves. In future, the methods utilizing cells, blood–brain barrier (BBB) models or brain slice cultures may improve accuracy in forecasting nanotoxic effects in the central nervous systems (CNS) of animals.

At first, our group investigated cell-based evaluation models, using strains of human neural stem cells or primary cultures of mouse cerebral cortex cells. A silica particle's co-culture system detected concentration-dependent abnormalities in cell morphology. In addition, this system with various nanoparticles had better sensitivity and specificity in detecting those cell morphology abnormalities than toxicology results obtained by the conventional mitochondrial activity tests.

Secondly, BBB models (rat central nervous cells) showed that the size and surface modification of the nanoparticle regulated its membrane permeability. These results not only were parallel with *in vivo* post-administration works of nanoparticles to the animals, but also suggested that the toxicity itself would be modifiable by engineering sizes and surfaces of the particle.

The third method is a brain slice culture; the sliced tissues were co-cultured with titanium dioxide nanoparticles. The mRNA-expression fluctuations in these tissues were analyzed with a next-generation sequencer, analyzing cDNA molecules reverse-transcribed from the mRNA of the original culture. The expressed genes indicated the candidate gene clusters of cellular death and cognitive disease-related genes. At the same time, this method does not predict quantities of the respective protein production.

In this presentation, we discuss the utility of these evaluation models, the efficacy of the experimental results of the cell-based assessment methods, and how to interpret, analyze and communicate the values and possible discrepancies of these assessment measurements effectively with other CNS toxicity testing models.



Central nerve models utilized for the nanotoxicity assessments

N°O3c-4

**COMPARATIVE STUDY OF NEUROLOGIC EFFECTS OF NANO-TiO₂ VERSUS SiO₂
AFTER DIRECT INTRACEREBRAL EXPOSURE IN MICE**

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The expanding development and production of engineered nanomaterials (ENMs) cover varied and extensive potential benefits in consumer products, food, drugs... The unique properties of ENMs have also raised concerns about the potential non intended consequences on human health and the environment. A potential risk for neurotoxicity arises if exposure leads to systemic absorption and distribution in the nervous system. However, neurotoxicity remains poorly documented. Thus, to consider whether any brain exposure may trigger a neurotoxic effect we developed a first approach based on direct injection of nanoparticles (NP) into the brain of C57Bl6 mice using a stereotaxic apparatus. We compared the neurologic effects of a single injection of nano-TiO₂ and nano-SiO₂ on motor performance using a rotarod equipment. Before and after injection motor activity is registered individually for each mouse exposed, once a week, for 8 weeks. Besides, mice are culled at 1, 2, 3, 4 and 8 weeks after exposure in order to study the time dependant effect on the histopathology of the brain (gliosis, inflammatory process, ...).

Nano-TiO₂ and -SiO₂ were selected because they are probe particles with positive (TiO₂) and negative (SiO₂) surface charge in biological media (pH 7-8). Both TiO₂ (aeroxide TiO₂ P-25, spherical 22 nm, 85% anatase, 15% rutile) and SiO₂ (primary particle of 5-35 nm, specific surface 220 m²/g), are provided by the EC-JRC in the framework of the OECD sponsorship program as representative nanomaterial for research purposes. The dispersion protocol developed by Nanogenotox Joint Action was used in the present study. This generic Nanogenotox dispersion protocol determined to harmonize and standardize the dispersion of ENMs, produces ENM exposure media suitable for *in vitro* and *in vivo* toxicity testing. Injections were performed under deep anesthesia into the ventricular compartment of the brain in order to favor the diffusion of NP into the entire brain. The injection site was checked histologically. We followed the local needle scar and the histological recovery of the injury in a time dependant manner. Neurotoxic effects were investigated using motor performance parameters, measured on a rotarod at 20 rpm or at an accelerating rod (from 4 to 40 rpm).

Results showed that, whereas control mice (before injection) run at least 129 s +/- 105 (means +/- SEM) on the fixed rotarod, nano-sized SiO₂ exposition induced a deficit in motor performance as soon as 1 week after surgery (29 s +/- 52 *p<0.05). Using an accelerated rotarod, performances were also early significantly reduced at 1, 2 and 3 weeks after surgery compared to control mice (*p<0.05). Nano-TiO₂ induced a more progressive deterioration of each motor performance (*p=0.0001). At 20 rpm the initial performance of 104 s +/-86 decline slowly to 84 +/- 100 at 1 week and became null after 4 weeks. At 1 week, a local gliosis with an inflammation was seen at the initial point of injury as showed by astrocytes and microglial cells activation. Obviously this was not linked to the NP themselves. Thereafter, whereas gliosis progressively vanished, microglial activation grew throughout the brain suggesting an induction of a long lasting neuroinflammation, most probably linked to the NP. This fitted well with the loss of motor performances. In conclusion, our preliminary study shows that nano-sized SiO₂ and TiO₂ are both able to induce some neurological effects after direct exposure into the brain as shown by loss of motor performance of exposed mice.

N°O3c-5

**INTERACTIONS OF FUNCTIONALIZED MWCNTS WITH PRIMARY NEURAL CELLS FROM
DIFFERENT BRAIN REGIONS: SITE-SPECIFIC EFFECTS**

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High surface tunability and ability to penetrate cells make carbon nanotubes (CNTs) promising intracellular delivery systems for pharmaceutical or diagnostic purposes in the central nervous system (CNS). Proof-of-concept studies have also demonstrated their potential to mitigate ischemic damage in the brain. But before using them more widely as neural nanovectors, CNTs must demonstrate their biocompatibility with the different neural cell populations constituting the different brain regions. The goal of the present study was to determine the biological impact of different types of chemically functionalized (carboxylated or aminated) or pristine multiwalled CNT (MWCNT) on the neuronal and glial cell populations isolated from two foetal rat brain area, namely the frontal cortex (FCO) and the striatum (ST). Neurons from both brain regions were not affected by any exposure to MWCNTs as determined by a modified LDH assay. On the contrary, the viability of mixed glial cells (composed mainly of astrocytes and microglial cells) was reduced in ST-derived mixed glial cultures but not in the FCO-derived cultures. The cytotoxicity was also independent on the MWCNT type (*i.e.* pristine, carboxylated or aminated), suggesting an inherent sensitivity of the ST-derived mixed glial cultures compared to FCO-derived equivalent when exposed to MWCNTs. Characterization by immunocytochemistry of non-exposed mixed glial cultures showed a higher number of CD11b/c positive cells (indicative of microglia presence) in the ST-derived mixed glial cultures compared to their FCO-derived equivalent. Moreover, a higher degree of CNT uptake was evident in CD11b/c positive cells (microglia) compared to GFAP positive cells (astrocytes), for both regions. Such results suggested that the differential toxicity observed in ST-derived mixed glial culture is highly dependent on the higher number of responsive microglia cells. This was further demonstrated by the dose-dependent toxicity observed in microglia enriched cultures derived from both ST and FCO regions. These findings emphasized the role of the microglial cells, resident macrophages of the CNS, in response to nanomaterials and stress the need to study the region specific effects when dealing with brain delivery using nanomaterials.

N°O3c-6

SPECIFIC UPTAKES AND DAMAGES INDUCED BY POLYSTYRENE NANOBEADS ACCORDING TO SURFACE CHEMISTRY

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It is postulated that surface chemistry clearly modulates and impacts nanoparticles toxicity. On the other hand, data are missing regarding the impact of nanoparticles functionalization and their effects on human cells. Furthermore, even though inhalation is one of the major routes of exposure to nanoparticles, up-to-date, no evaluation of genotoxic effects of polystyrene (PS) nanobeads on human lung has been published. For this reason, we decided to study cytotoxicity and genotoxicity effects of PS nanobeads on Calu-3 pulmonary epithelial cells and THP-1 macrophages jointly with cell-uptake. This study was performed with mono-dispersed and perfectly characterized fluorescent 50nm PS nanobeads, harboring different functionalized surfaces: non-functionalized (PS-NF), carboxylated (PS-COOH) and aminated (PS-NH₂). Cytotoxic effects were measured by xCELLigence system (24h real-time monitoring) and AlamarBlue® assay (2, 4 and 24h), while genotoxic effects of both nanoparticles were evaluated through γ -H2Ax-foci detection method (2, 4 and 24h). Nanobeads uptake for each cell line was assessed by video-confocal microscopy 24h monitoring. All together our results clearly report that PS-NH₂ nanobeads are able to induce cell death for both cell lines, while PS-NF and PS-COOH do not lead to significant cytotoxic and genotoxic effects. Besides impacting their biological effects, PS nanobeads surface chemistry seems to clearly impact their internalization (Fig. 1). Indeed, PS-NH₂ nanobeads are able to induce cell death and DNA double strand breaks while it is the less incorporated by cells among the three tested. In conclusion, our results have enlightened that for similar concentrations PS-NH₂ nanobeads are more cytotoxic and genotoxic than PS-NF and PS-COOH for both tested cell lines whereas they were less incorporated than each other tested nanobeads. These results strongly support the primordial role of nanoparticles surface chemistry on their internalization and their biological effects upon human cells.

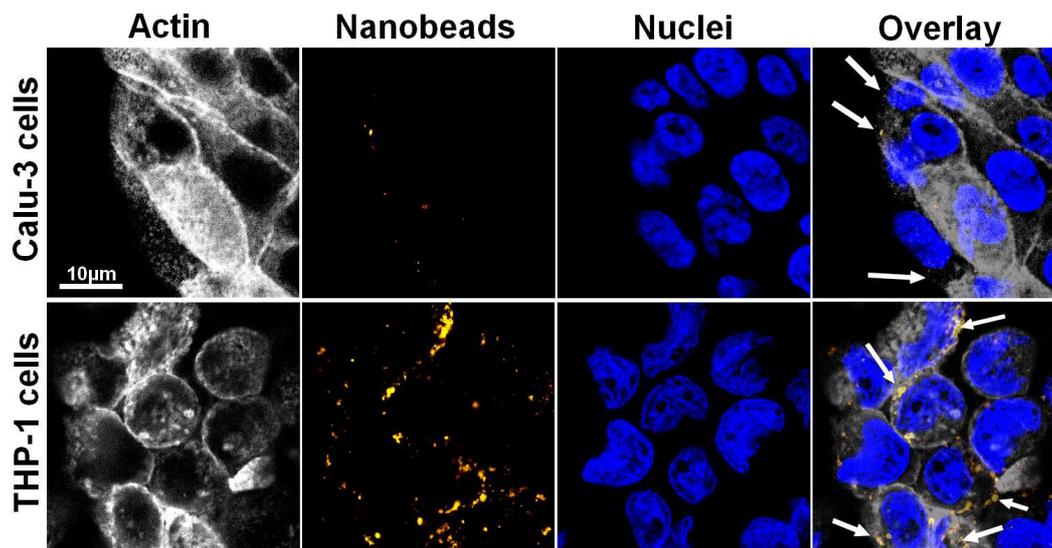


Fig.1. Confocal microscopy detection (40X) of PS-NH₂ nanobeads in Calu-3 cells and THP-1 macrophages after 4h of exposure (actin is labeled in grey (Alexa Fluor® 635 Phalloïdin), nanobeads in yellow/orange and nuclei in blue (Hoechst 33342)). Nanobeads (indicated by white arrows on overlay slides) are mainly located on the cytoplasm for THP-1 macrophages, while they are slightly incorporated and mainly located in periphery cells islets for Calu-3 epithelial cells.

N°O3c-7

APPLICATION OF *IN VITRO* BBB MODEL TO MEASURE PERMEABILITY OF NANOPARTICLES

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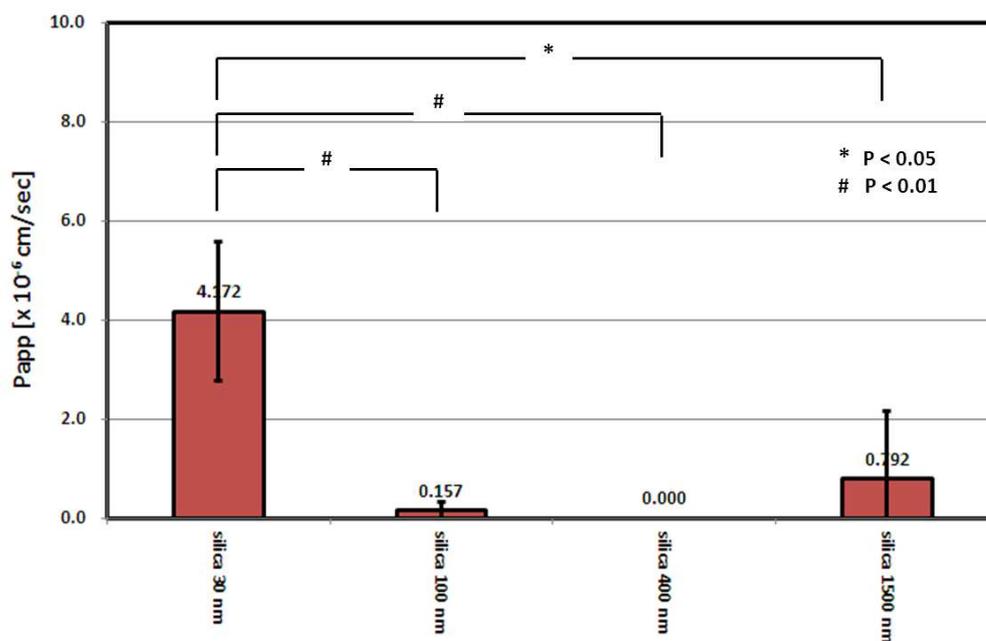
In both pharmaceutical and toxicological fields, one of major issues has been the possibility of nanoparticle uptake to central nerve system. Recent reports showed that 70 nm silica accumulated brain endothelial cells, some of nanoparticles passed through the blood-brain-barrier (BBB), and stayed in brain parenchyma. For the safe use of nanoparticles, it is integral to evaluate the permeability of nanoparticles through BBB.

In this study we applied *in vitro* BBB model to quantify the permeability of nanoparticles for rapid, easy and highly reproducible assay. The *in vitro* BBB model (PharmaCo-Cell Company Ltd, Japan) is reconstructed with culturing both primary rat brain endothelial cells and pericytes separated by transwell membrane, which are pre-cultured with astrocytes to support the tight junctions of endothelial cells. We examined the size-dependency of the BBB permeability, using fluorescent silica nanoparticles (30 nm, 100 nm, 400 nm and 1500 nm) and the surface modification effects using quantum dots (amino-, carboxyl- and PEGylated-QDs). Permeability of nanoparticles is evaluated by permeability coefficient (Papp).

The size-dependency results found 30 nm silica transports through BBB model and the Papp is 4.272, which is the level of drugs known slightly passable through the BBB model, while the nanoparticles with the size of 100 nm or larger hardly passed through the BBB model (0.157, 0, 0.792). The fluorescence of the nanoparticles confirmed only 30 nm nanoparticles accumulated in the both sides of layers with endothelial cells and pericytes.

The surface charge-dependency revealed amino-nanoparticles passed through the BBB model more than carboxyl- and PEGylated-nanoparticles, but the results had discrepancy with other *in vivo* works. The reason can be that protein-free buffer in our BBB model worked differently from *in vivo* serum that adsorb to nanoparticles, e.g. “protein corona”.

In conclusion, we showed the *in vitro* BBB model could be established as a method to test quantifiable permeability of nanoparticles. In future, we plan further improving this model to fit the *in vivo* environment.



Size-dependent BBB permeability of silica nanoparticles

N°O3c-8

3D MODEL OF AIR-BLOOD BARRIER FOR THE STUDY OF NANOPARTICLES TRANSLOCATION

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The increasing use of nanoparticles in numerous industrial sectors leads us to evaluate their potential risk for human health. As the lung is one of the main routes of exposure to NPs, we developed an *in vitro* model of air-blood barrier to study nanoparticles translocation and potential changes on barrier integrity.

In order to mimic the alveolo-capillary barrier, the model is composed of 3 cell types (Figure 1): macrophages (PMA-differentiated THP-1 cells), lung epithelial cells (Calu-3) and pulmonary endothelial cells (HPMEC-ST1.6R), cultivated on apical side (macrophages + epithelial cells) and on basal side (endothelial cells) of a microporous membrane. As the number of macrophages within the alveoli is highly variable among individuals, we used different concentrations of macrophages in our model.

We established well-characterized models with high trans-electric epithelial resistance (TEER) values similar to primary human alveolar epithelial cells ($>1000\Omega\cdot\text{cm}^2$, 7 days post seeding). Adherent and tight intercellular junction proteins (E-cadherin, VE-Cadherin and Occludin) were observed in co-cultures. Low macrophage concentrations (*i.e.* 1 or 2×10^4 cells/Transwell®) had no effects on TEER values, contrary to the highest concentration (*i.e.* 5×10^4 cells/Transwell®), which decreased TEER values from 30%. At the day 8, bi- and tri-cellular models (without or with macrophages respectively) were exposed to fluorescent non-functionalized (50 and 100nm) or aminated (50nm) polystyrene (PS) nanobeads during 24h. No cytotoxicity was measured for all tested nano-beads, while we previously reported that only aminated PS nanobeads were cytotoxic for each cell type cultivated alone. Interestingly, 50 and 100nm non-functionalized PS nanobeads prevent increase of TEER compared to control cells in all co-cultures after 24h exposure while aminated PS nanobeads had no significant effect. In all models, internalization and translocation were only detected for non-functionalized 50nm PS nanobeads and translocation was independent on macrophage concentrations.

These results point out the importance of using co-culture models to assess *in vitro* effects of NPs and confirm the role of size and surface chemistry in the behavior of NPs within the air-blood barrier.

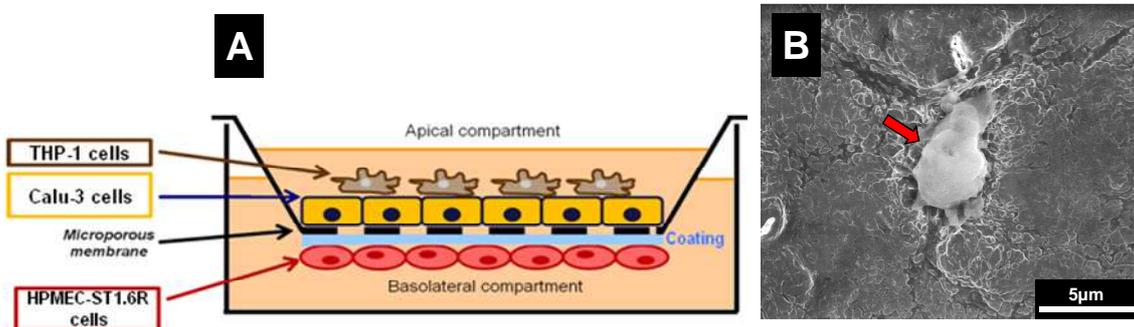


Figure 1. (A) Representation of the model of alveolo-capillary barrier *in vitro*. THP-1 macrophages, Calu-3 epithelial cells and HPMEC-ST1.6R endothelial cells are co-cultivated on both sides of a Transwell®. (B) SEM picture showing an activated macrophage with pseudopodias (red arrow) on the apical side of Calu-3 epithelial cells.

N°O3c-9

ASSESSMENT OF CYTOTOXICITY, INTRACELLULAR UPTAKE AND INTESTINAL ABSORPTION OF AMORPHOUS SILICA NANOPARTICLES IN THE CACO-2 IN VITRO HUMAN INTESTINAL BARRIER MODEL

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The Scientific Committee of the European Food Safety Authority has issued a recommendation stating that specific actions should be taken to generate information on the toxicity of different engineered nanomaterials found in food (EFSA, 2011). Amorphous silica (SiO₂) nanoparticles (NPs) are widely used in many biomedical and industrial applications, including cosmetics, food and oral medicines. The food additive (E551) commonly used in many food products, can also contain SiO₂ in nano-sized form (Dekkers et al. 2011). Although available acute and repeated-dose in vivo studies have not shown toxicity of SiO₂ NPs after oral exposure, up to now no detailed information is available on the absorption of SiO₂ nanoparticles from the gastrointestinal tract.

In the present study the toxicological profile, the intracellular uptake and passage of SiO₂ NPs across the intestinal barrier was assessed in in vitro for the Caco-2 cells. These cells grown on permeable inserts form a polarized epithelial cell monolayer that provides a physical and biochemical barrier to the passage of ions and small molecules, and are therefore widely used as a model of human small intestinal mucosa.

The cytotoxicity of SiO₂ (15, 35 and 85 nm-sized) in Caco-2 cells was assessed using the Colony Forming Efficiency assay. All three forms of SiO₂ NPs did not induce cytotoxicity, neither after a single (acute), nor after a repeated-dose exposure. Moreover, Caco-2 cells were differentiated on permeable inserts, exposed to repeated doses of SiO₂ nanoparticles over a period of 21 days and the potential damage to the epithelial barrier was evaluated. No significant changes in trans-epithelial electrical resistance (TEER) and Lucifer Yellow permeability were found, indicating that the Caco-2 barrier was not compromised after exposure to SiO₂ NPs.

The uptake and the intracellular localization was studied using 85 nm-sized fluorescent amorphous silica NPs, labelled with Tris(2,2'-bipyridyl)-dichlororuthenium(II) hexahydrate (Ru(II)(bipy)₃Cl₂) and a panel of markers was used for the endosomal pathway and mitochondria (Uboldi et al, 2012). The SiO₂-Ru(II)(bipy)₃Cl₂ NPs showed no toxicity, were quickly (after 1 h) taken up by the cells and stored in vesicles located in the perinuclear region. Biokinetic studies on intestinal absorption of SiO₂ NPs were performed in Caco-2 cells grown on permeable inserts, by measuring the passage of radiolabelled ⁵⁶Co-SiO₂ NPs 20, 40 and 90 nm sized (Cydzik et al, 2012) across the epithelial barrier over time (2-72 h). A small (~1%) passage of SiO₂ NPs was observed after 24 hours and only slightly increased in time (~4% after 72 hours).

In conclusion, SiO₂ nanoparticles did not induce cellular toxicity and damage to the Caco-2 epithelial barrier, but were taken up by and accumulated in the cells in a time-dependent manner. However, they were not transported across the Caco-2 barrier in large amounts, indicating that their intestinal absorption is very low.

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N°O3c-10

**INFLUENCE OF THE LENGTH OF IMOGOLITE-LIKE NANOTUBES ON THEIR CYTOTOXICITY
AND GENOTOXICITY TOWARDS HUMAN DERMAL CELLS**

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Physical-chemical parameters as purity, structure, chemistry, length and aspect ratio of nanoparticles (NPs) are linked to their toxicity. Here, synthesis imogolite like nanotubes with constant chemical composition but various sizes and shapes were used as models to investigate the influence of these physical parameters on the cyto-genotoxicity and cellular uptake of NPs. NPs synthesis were characterized using X-ray diffraction (XRD), Small Angle X-ray Scattering (SAXS), Atomic force microscopy (AFM). Imogolite precursors (PR, ca. 5 nm curved platelets), as well as short tubes (ST, ca. 6nm) and long tubes (LT, ca. 50 nm) remained stable in the cell culture medium. Internalization into human fibroblast was observed only for the small particles PR and ST. None of the tested particles induced significant cytotoxicity up to concentration of 10⁻¹ mg.mL⁻¹. However, small sizes NPs (PR and ST) were found to be genotoxic at very low concentration 10⁻⁶ mg.mL⁻¹, while LT particles exhibited a weak genotoxicity. Our results indicate that small size NPs (PR, ST) were able to induce primary lesion of DNA at very low concentration, and this DNA damage was exclusively induced by oxidative stress. The higher aspect ratio LT particles exhibited a weak genotoxicity, oxidative stress has a minor effect in genotoxicity and other mechanisms are probably involved. Moreover, a relationship between cell uptake, particle aspect ratio and DNA damage of NPs was observed.

N°O3c-11

EFFECT OF DIFFERENT SURFACE CHARGE BASED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPION) ON BIODISTRIBUTION IN RAT AND EX VIVO PROTEIN FISHING

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There has been extensive research into the applications of nanoparticles (NP) in areas such as cosmetics, foods and medicine. There is an urgent need for understanding their fate within anatomical structures of living systems, especially individual compartmentalization. Nanoparticle properties (such as size, surface chemistry, and composition) affect the physiological processes of nanoparticles involving opsonization in the blood, biodistribution, cellular recognition and internalization, enzymatic degradation and changes in physical properties. Protein adsorption to nanoparticle surfaces, termed opsonization, occurs almost instantaneously once the particle enters the blood circulation, and the physicochemical properties of these nanoparticle-protein complexes are often different to those of the original nanoparticle. The protein adsorption onto nanoparticles will further determine the fate of NP in physiological system. In this work, the great efforts have been made to investigate the effect of different surface polymer charge coated SPION on the correlation of protein adsorption and biodistribution. The 3 different surface charge charged Polyvinyl Alcohol (PVA) polymer coated SPION (positive, neutral and negative) were intravenously injected into rats. The rats were sacrificed 15 minutes after nanoparticle injection for all nanoparticles and 7 days for positively charged nanoparticles. More than 10 organs, including blood, were collected for biodistribution studies by the titration technique. Sera were collected for an *ex vivo* protein fishing by using a magnetic reactor, SDS-PAGE and Mass Spectrometry (MS). The results show that, after 15 minutes of injection, all 3 particle types are located mainly in serum and liver. The positively charged nanoparticles were localized to the liver 4 times more than neutral and negatively charged nanoparticles. The concentrations of the latter two particle types were twice those of the positively charged particles in blood. In long term investigation of 7 days following injection, the positively charged nanoparticles concentration was significantly decreased in serum and slightly decreased in the liver; however, liver remained the main site of nanoparticle deposition. The amount of each type nanoparticle in serum that can be trapped by a magnetic reactor also correlated with the SPION titration result by magnetic susceptibility. For protein fishing, the nanoparticles in serum were magnetically trapped in a magnetic reactor before salt elution of the nanoparticle-adsorbed protein. SDS-PAGE revealed tightly bound bovine serum albumin. A detailed study of other tightly bound proteins will be confirmed by MS analysis.

N°O3c-12

**AN IMPEDANCE-BASED HIGH-THROUGHPUT METHOD FOR EVALUATING THE
CYTOTOXICITY OF NANOPARTICLES**

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Impedance-based assays can constitute a reliable alternative to the conventional methods used in nanotoxicology due to the important advantages of being label-free and monitoring the cells in real-time. They require no markers or reagents, which generally tend to interfere with the method of detection and thus significantly distort the results. In this study, the suitability of impedance-monitoring for the screening of nanoparticle (NP)-induced cytotoxicity was assessed.

The effect of TiO₂-NPs on cellular proliferation, viability, morphology, spreading, and detachment from substrate were evaluated by continuous impedance-based measurements made with the xCELLigence System (Roche Applied Science, Mannheim). Two spherical anatase TiO₂-NPs, 5 and 10 nm in diameter, were characterized by X-ray diffraction, transmission electron microscopy (TEM), N₂-BET adsorption, dynamic light scattering, and electrophoretic mobility. Fibroblasts were seeded in microelectrode-embedded micro-well –E-plates inserted into a real-time cell electronic sensing station which was placed inside an incubator. Cells attached to the electrode sensor surfaces act as insulators leading to an increase in impedance. The cells were exposed to 0.05, 0.5, and 5 mg/L nano-TiO₂ for up to 120 h. The measurements were made by sending an AC signal (20 mV voltage amplitude) at three different frequencies (10, 25 and 50 kHz) through the microelectrodes in the E-plates and then measuring the resulting impedance. The software displays the measured impedance as a Cell Index (CI) for each point in time. A change in CI can be indicative of changes in cell number, cell adhesion, spreading, or cell morphology. For validating the impedance-method, ultrahigh resolution imaging (URI) microscopy (CytoViva™, Auburn, TX) and trypan blue exclusion were employed.

The general trend observed was a decrease in impedance following exposure to TiO₂-NPs. Cytotoxicity increased with concentration. Cellular modifications indicative of apoptosis were observed by URI. Impedance-based results were in accordance with the findings from the trypan blue exclusion assay and URI imaging.

The results of our study indicate that impedance-based methods are reliable tools for real-time monitoring of cells exposed to NPs and for cytotoxicity assessment.

Acknowledgments: Zouhir E. Allouni for NP characterization, Richard Davies for impedance measurements, and for financial support: the NanoUiB platform, the Dept. of Clinical Dentistry, Fac. of Medicine and Dentistry, Univ. of Bergen, and the Christian Michelsen Research Inst., Bergen, Norway.

N°O3c-13

THE USE OF DIFFERENTIATED HUMAN RESPIRATORY EPITHELIAL CELLS IN INHALATION TOXICOLOGY OF NANOMATERIALS

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Predictive *in vitro* tests are needed to rank nanomaterials (NM) according to toxicity and bioavailability, in order to determine priority for subsequent *in vivo* testing. Human 3D airway models allow the investigation of functional endpoints like ciliary clearance, and the role of mucus in nanoparticle behaviour. The 3D model consists of fully differentiated human respiratory epithelial cells (basal, ciliated and goblet cells) with intact metabolism functionalities, covered by a mucus layer. To investigate the applicability of human 3D airway models in the safety assessment of NM, we tested the toxicity of SiO₂ and CeO₂ nanoparticles on MucilAir™ (EpiThelex Sàrl). We selected this model to study (geno)toxicity, because many substances induce histopathology of the respiratory epithelium, despite the fact that the respiratory epithelium represents a much smaller surface area than the alveolar epithelium. The 3D model was exposed for 3, 24 and 48 h to a dispersion of the NM that was applied on the tissue surface (droplet exposure). Cytotoxicity was measured by lactate dehydrogenase (LDH) and transepithelial electric resistance (TEER). Various cytokines were analysed in culture medium as a measure for inflammation, and genotoxicity was evaluated by the comet assay. No effect was measured on LDH, TEER and the pro-inflammatory cytokines. A slight increase in percentage tail DNA was observed with cerium oxide, at 3 h after exposure. This response was considerably less than observed with cerium oxide in cultures with RAW 246.7 cells. Interaction of NM with mucus, DNA repair and apical exposure versus exposure via immersion all contribute to the observed difference in response. In general, the results are considered to be a realistic response against exposure to the tested NM.

Acknowledgement: NanoNextNL, a consortium of the Dutch Government and partners.

N°O3d-1

ROS EVALUATION FOR A SERIES OF CNTS AND THEIR DERIVATIVES USING ESR METHOD WITH DMPO

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Carbon nanotubes (CNTs) have been applied for industrial products such as energy devices and resin composite, and their new applications with unique characteristics are actively proposed in the different fields of technologies. On the other hand, it has been reported that CNTs induce lesions in *in vitro* and *in vivo* tests such as cancer and mesothelioma, which indecisively slows down CNTs major progresses in industries. Recently, several reports point out that CNTs do not generate reactive oxygen species (ROS) and rather scavenge ROS depending on CNTs properties: diameter, length, crystallinity, defect, functional groups attached, and included impurity species and concentration in CNTs. To elucidate the biological reactivity of CNTs, the ROS evaluation with ESR might become a good indicator to determine the intrinsic physicochemical properties in relation to their biological activities. It is deduced from the conventional toxicology in which toxicological reactions in a living body are induced by chemical reactions with electron transfer between CNT surface and molecules of protein, DNAs, and/or macroscopically cells. Although the electron charge transfer seems one of the noticeable phenomena of toxicological chemical reactions, any comprehensive evaluation on ROS scavenging capabilities of CNTs has not been exhibited well. The present work specifically investigates ROS scavenging capabilities using the series of cup-stuck type CNTs (cs-CNTs) and their derivatives. Since this particular type of CNTs has many carbon edges on the surface and can be controlled surface conditions of those dangling bonds by heat treatment and metal dope, the reaction mechanism is sought through hydroxy radical scavenging. Those ROS scavenging properties were measured by electron spin resonance (ESR) with 5, 5-dimethyl-1-pyrroline-N-oxide (DMPO). It is highlighted that those CNTs highly crystallized at 2800°C, mechanically chopped, mechanically de-bulked, and Platinum nano particle doped to the highly crystallized one were evaluated (Group A). Furthermore, seven kinds of commercially available CNTs (Group B) were compared with cs-CNTs. It was interesting that the ROS scavenging rate was not significantly influenced by those mechanical treatment procedures, but that it was decreased in comparison with the original cs-CNTs by crystallization at high temperature. Also, ROS scavenging rate was increased by Pt-doped CNTs compensating the high crystallinity. Although the cs-CNTs have a lot of carbon edges or dangling bond, the scavenging rate of the original cs-CNTs and those of Group B CNTs but a particular CNT showed no significant difference. One of Group B CNTs significantly exhibited the lower ROS scavenging capability though it did not generate ROS at all. Those results imply that the electron transfer on the CNT surface is the fundamental mechanism to explain the ROS scavenging. Dangling bonds generated mechanically are not effective. Thus, ROS property for CNTs is determined based on the intrinsic properties of CNTs depending on the manufacturing processes.

N°O3d-2

TOXICITY TOWARDS LUNG CELLS AND ESCHERICHIA COLI: IMPACT OF NANOPARTICLE DISPERSION STATUS

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The toxicity of engineered nanoparticles (NPs) is of interest in the frame of development of devices based on nanotechnology. However, most common toxicity assays require dispersion in biological compatible media and NPs tend to agglomerate in such media (LB, DMEM...). Therefore, efforts have been done in the past few years to achieve dispersion as good as possible while remaining in relevant exposure conditions. In particular, several protocols now recommend using high power ultrasonic dispersion before exposure experiments (for instance, see recommendation from OECD sponsorship program on nanomaterials). In the present study, we show that the use of such high power devices may lead to the observation of toxic effects which are not necessarily related with the state of NPs dispersion.

For such study, two different systems, prokaryotic or eukaryotic cells, were studied: we have in particular studied the dispersion of a very common industrial titania NP (Degussa P25 produced in ton quantities). When dispersed in water, the suspensions of NP appear stable for weeks. When transferred in the cell culture medium (DMEM) or if directly dispersed in DMEM, strong evolution of size is seen as well as sedimentation. By contrast, when a protein-containing "surfactant", relevant with biological studies (Foetal Bovine Serum (FBS)) is added in the suspension in order to coat the nanoparticles prior to transfer in DMEM (or other cell media), the dispersion appears stable with limited agglomeration. Therefore, the studies are dealing with agglomerates or isolated nanoparticles and the effects are different. Moreover, we have compared different dispersion methods, coming from materials science, of dispersing NP in water with the idea to break in a preliminary step some of the necks between nanoparticles. The effect of dry ball milling, liquid ball milling and ultrasonic dispersion are compared. It is shown that for both eukaryotic and prokaryotic cells models, although the dispersion is quite similar, from a size dispersion point of view, with ultrasonic probe or ball milling, the toxicity appears very different according to the dispersion method, i.e. the suspension obtained after dispersion using ultrasonic device appears more toxic than using mechanical dispersion and similar trends are observed for both eukaryotic and prokaryotic systems.

N°O3d-3

A NOTEWORTHY INTERACTION OF TiO₂ NANOPARTICLES (ANATASE) WITH BACTERIAL CELLS UNDER DARK CONDITIONS

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The cytotoxic potential of TiO₂ nanoparticles (NPs) under dark conditions has not been well explored since most of the prior cytotoxicity studies focused on irradiated TiO₂. Only a few researchers have thrown light on TiO₂ NP toxicity under dark conditions towards bacteria. A possible involvement of free radicals has been demonstrated as one of the underlying mechanism. On the other hand, physical interaction of NPs with cells has been hypothesized to be a contributory factor. The current study presents a direct evidence of fundamental mechanism of cell–NP interaction in the absence of photoinduction. The interaction of a dominant freshwater bacterial isolate, *Bacillus licheniformis*, at environmentally relevant concentrations of TiO₂ NPs (1 µg/mL) was studied. Filtered freshwater was used as the experimental matrix. A statistically significant reduction in cell viability was observed under dark conditions. Though the NPs were found to be stable against aggregation for a period of 24 h, an increased rate of sedimentation was observed in the presence of bacterial cells, in the absence of light. Oxidative stress was found to play an important role in causing cytotoxicity through ROS assessment. NP adsorption onto the cell membrane and its internalization observed through electron microscopy (SEM, TEM) and quantified through ICP-OES analysis was found to be the dominating factor for cell damage and death. The FT-IR and FT-Raman spectra confirmed participation of cell surface functional groups like Amide–I, Amide–II and carbonyl groups, in the interaction between cell surface and the nanoparticles. The electron paramagnetic resonance (EPR) spectroscopy substantiated the reduction of titanium from Ti⁴⁺ to Ti³⁺ confirming the involvement of redox interactions on the cell surface. This study points out to the crucial role of interfacial interactions in cytotoxicity of TiO₂ NPs under dark conditions ruling out the solitary inevitable contribution of photoinduction.

N°O3d-4

**DETERMINATION OF ENDOTOXIN CONCENTRATION BY DIFFERENT TEST METHODS:
INFLUENCE OF SAMPLE PREPARATION AND PARTICLE INTERFERENCE ON TEST
RELIABILITY**

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Endotoxins (lipopolysaccharides, LPS) are part of the outer membrane of the cell wall of gram-negative bacteria. It cannot be excluded that nanomaterials are contaminated with endotoxin during production or handling. Endotoxin contamination should be assessed when evaluating the potential toxicity, to distinguish specific nanoparticle toxicity from the endotoxin effects. Currently, the limulus amoebocyte lysate (LAL) test is the assay of choice for the determination of endotoxin.

The aim of our study is to find a convenient *in vitro* test method to evaluate endotoxin contamination in particle samples.

We assessed the reliability of different LAL assays – gel clot, chromogenic and a FDA-licensed endotoxin detection system (Endosafe-PTS) – when performed in the presence of nanoparticles, as well as an endotoxin extraction method as described in the ISO norm on endotoxin test on nanomaterial samples for *in vitro* systems. Another endotoxin test method based on Toll-like receptor (TLR) 4 reporter cells was evaluated as well.

Our results indicate that at least one LAL-based assay – the gel clot LAL assay – and the endotoxin extraction protocol are not suitable for evaluating endotoxin contamination in nanoparticle formulations. No interfering effects were reported applying chromogenic-based LAL endotoxin detection systems (chromogenic LAL assay and Endosafe-PTS). Furthermore, TLR4 reporter cells form a more convenient and reliable alternative for measuring endotoxin contamination in general, and more specific in nanoparticle formulations.

Sponsor

EU-FP7: NanoHouse

N°O3d-5

**VIOLACEIN/POLY- ϵ -CAPROLACTONE/CHITOSAN NANOPARTICLES AGAINST BOVINE
MASTITIS: ANTIBACTERIAL AND ECOTOXICITY EVALUATION**

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Bovine Mastitis, one of the most important bacterial infections in livestock, is responsible for heavy economic loss. Therefore new treatment approaches should be attempted. The aim of this study is to encapsulate violacein (Fig.1 a,b), a purple pigment from *Chromobacterium violaceum* with antibactericidal properties. Polymeric nanoparticles are attractive carriers to lipophilic compounds as violacein because they can increase efficiency and stability. To inhibit the adsorption of the carriers by cationic proteins present in biological fluids and increase the interaction with cell membrane a charge surface study was carried out. The nanocarrier was synthesized by nano-precipitation, using poly- ϵ -caprolactone (PCL) as polymer, Tween-80 as surfactant and the biopolymer chitosan (CS) as a charge modification agent. Charge, size and morphology were analyzed using Zeta Potential, photo correlation spectroscopy, scanning electron microscopy (SEM) and differential scanning calorimetric (DSC). Bactericidal assays were carried out using a resistant strain of *Staphylococcus aureus*. Acute ecotoxicity tests were performed with *Daphnia similis* (Fig. 1 c). The Zeta potential and size analysis with Tween 80 (1 - 3 mg/ml) and the CS (0 - 1 mg/ml) were measured. Both, Zeta potential and size were influenced by these variables, but the encapsulate efficiency was not. Two systems one containing 1 mg/ml of Tween and 0 mg/ml of CS (PCLnpVio) and other with 0.425 mg/ml of CS (CS/PCLnpVio) encapsulate 100%, with 10% of drug loading were prepared. DSC analysis showed that violacein can be dissolved in polymer matrix. The PCLnpVio system exhibited an average size of 78 ± 4 nm and Zeta potential of -4.28 mV, and the CS/PCLnpVio showed 250 ± 80 nm, +21.3 mV, stable for 30 days. The minimal inhibitory concentration against *S. aureus* of CS/PCLnpVio was <25 μ M, while for PCLnpVio and pure violacein were >25 μ M. Both nanoparticles presented an EC₅₀ of 1.5 nM to *Daphnia*, two times more toxic than violacein itself. We described the possibility to control the charge of the nanoparticles, without extreme change in size and that it is possible also to encapsulate a powerful antibactericidal compound such as violacein. Attention should be given to the relative high toxicity to microcrustaceans when environmental exposures are expected.

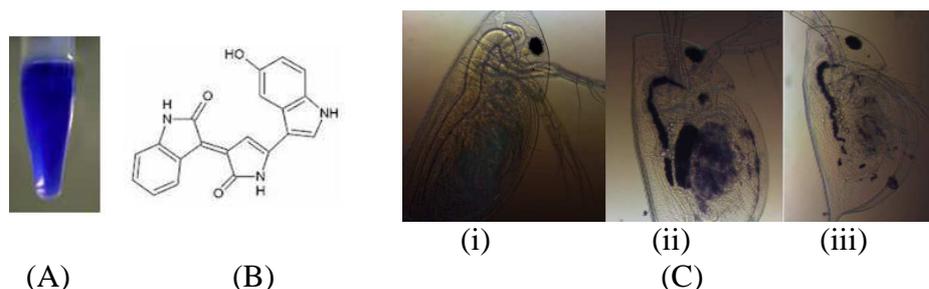


Fig.1. (A) Violacein solution in ethanol, (B) violacein structure, (c) *Daphnia* control (i), and different stages with violacein (ii) and (iii).

Acknowledgement: CNPq, FAPESP, INOMAT (MCT/CNPq) and Brazilian Network Nanotoxicology (CNPq)

N°O3d-6

EVALUATION OF MULTI WALLED CARBON NANOTUBES ECO(GENO)TOXICITY USING THE AMPHIBIAN LARVAE OF *XENOPUS LAEVIS*

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Present knowledge concerning the ecotoxic effects of Carbon nanotubes (CNTs) is rather limited and deserves to be documented more extensively. Indeed, CNTs which are a special category of nanomaterials, with exceptional characteristics, are aimed to be used in numerous potential applications. In this context, their annual world production capacities continue to growth. Therefore, it is likely that some of them may contaminate the environment during their life cycle (production, use and disposal), especially in the aquatic compartment which is well known as the main receptacle of pollutants.

In this context, in the framework of the joint research laboratory NAUTILE (Nanotubes & Ecotoxicology), the potential toxicity of industrial Multi Walled Carbon NanoTubes (MWNTs) was investigated in amphibian larvae model (*Xenopus laevis*). MWNTs were synthesized by ARKEMA France (Graphistrength C100). The potential adverse effects of MWNTs were investigated in normalised laboratory conditions exposure (International Standard micronucleus assay ISO 21427-1) after 12 days of *Xenopus* exposure according semi-static exposure from 0.1 to 50 mg/L of MWNTs and after 24 h at 0.1 and 1 mg/L. Several endpoints were carried out: mortality, growth inhibition, micronuclei induction and primary DNA damages (micronuclei and comet assays) in erythrocytes of the circulating blood. Raman spectroscopy analysis was used to study the presence of MWNT in the biological samples.

After 12 days of MWNTs exposure, mortality and genetic effects (micronuclei induction and primary DNA damages) were not observed whereas growth inhibition was measured in larvae exposed to 50 mg/L of MWNTs. On the other hand, after 24h of exposure, significant DNA damages were recorded to lowest concentrations. Results suggest that MWNTs toxicity would involve indirect mechanisms leading to oxidative stress at short exposure time and do not lead to irreversible mutations since DNA repair occurred after 12 days of exposure. The observed toxicity could be in relation with MWNTs uptake *via* gill track and/or dietary route. However, dietary way would be the major route of MWNTs contamination, considering the large amount of MWNTs observed in the gut, even if MWNT were not identified in particular organs and cells using microscopy. Since MWNTs are ingested by larvae, the possibility that MWNTs may be found further in the food chain cannot be excluded, once released into the environment.

N°O3d-7

MOLECULAR INTERFERENCE BETWEEN TITANIUM FROM NP-TiO₂ NANOPARTICLES AND IRON HOMEOSTASIS IN *E. COLI*

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TiO₂ nanoparticles (TiO₂-NP) production has massively increased during the last decade as well as their use in commercial products. As a consequence, a major concern has been to address the potential toxicity of the TiO₂-NP on human health as well as on the environment. Studies using uncoated well-characterized TiO₂-NP have shown that these NP were cytotoxic, and induce an increase of the level of reactive oxygen species (ROS) in *E. coli* (1).

The purpose of our work was to investigate the molecular mechanisms leading to cellular effects of TiO₂-NP especially in relation with the potential disruption of iron homeostasis using *E. coli* as prokaryote model.

We firstly defined procedures to disperse the TiO₂-NP as well as to determine the exposure conditions in which our nanoparticles were well-dispersed in a specific cellular growth medium where a bacterial growth is still observed and not only a survival.

Secondly we studied in these define conditions the impact of a nanoparticle exposure on the bacterial growth and viability of several strains and mutants (in order to exacerbate phenotypes) on genes related to iron homeostasis and oxidative stress.

We also evaluated the localization of nanoparticles in presence of bacteria and quantify iron and titanium content using ICP-MS analyses.

In addition, we studied the solubilization and chelation of Ti(IV) from TiO₂-NP by enterobactin. The siderophores, such as enterobactin, are the physiological iron chelator involved in iron uptake in bacteria.

Finally a targeted study was performed to analyze the impact of nanoparticle exposure on enzymatic activities measured in cellular extracts. These targeted enzymes have metallic sites (iron-sulfur cluster) and can be potential biomarkers of a disturbance of the iron homeostasis.

We obtained original data that altogether suggest: i) titanium solubilization from the NP-TiO₂; ii) titanium entrance in the bacteria inducing a decreased in the iron content; iii) a loss of aconitase activity and the need of the SUF machinery in the response to the NP-TiO₂ stress.

Our data underlined how metallic nanoparticles could impact the metabolism of the bacteria without drastic effects on the viability. We can postulate that the small but significant effects observed on the metal (Fe and Ti) homeostasis could disrupt and induce evolution changes such as mutation and resistance acquisition on chronic and longtime interaction. It will now be interesting to study that point as well as to see if other nanoparticles can lead to the same kind of disturbance.

1. Simon-Deckers, A. et al. *Environ. Sci. Technol.* **2009**, 43, 8423–8429.

N°O3d-8

FULLERENE NANOPARTICLES C₆₀ AND C₆₀(OH)₁₈₋₂₂: ASSESSMENT OF BIOLOGICAL ACTIVITY USING BACTERIAL CELLS AND RAT LIVER MITOCHONDRIA AS MODEL SYSTEMS

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Fullerenes, commonly called buckyballs, are carbon nanoparticles with small size, large surface area and high reactivity. Since their discovery, fullerenes have been intensively investigated in order to develop formulations with specific biological activities to be used in different biomedical fields (from diagnosis methods to therapeutic applications). The size, shape, surface chemistry and surface charge have been identified as the physicochemical properties of fullerenes that can influence their interactions with biological systems. In the present study, water-colloidal dispersions of C₆₀ and C₆₀(OH)₁₈₋₂₂ were characterized by measuring the size and surface charge. The aqueous dispersion of C₆₀ was composed of nanoparticles with an average size of 32 nm, while C₆₀(OH)₁₈₋₂₂ dispersion nanoparticles have sizes lower than 2 nm. Both fullerenes acquired negative surface charge when dispersed in water. The influence of the different physical properties of these nanoparticles on their biological activity and toxicity were evaluated on two biological systems: bacterial cells (bacterium *B. stearothermophilus*) and rat liver mitochondria. At concentrations up to 20 mg/L (for C₆₀) and 200 mg/L (for C₆₀(OH)₁₈₋₂₂), the carbon nanoparticles showed to have no significant effects on growth of cultures of *B. stearothermophilus* in a liquid medium and on the ultrastructure of bacterial cells. Additionally, C₆₀ did not affect the respiratory activity of protoplasts of the bacterium (up to 200 µg/mg of cell protein), while its polar derivative [C₆₀(OH)₁₈₋₂₂] induced some perturbation, but only at concentrations higher than 900 µg/mg of cell protein. Studies on mitochondrial bioenergetics indicated that C₆₀ (up to 15 µg/mg of mitochondrial protein) stimulated the state 4 respiration and inhibited state 3 and FCCP-uncoupled respiration, supported either by glutamate/malate or succinate as respiratory substrates. On the other hand, C₆₀(OH)₁₈₋₂₂ (up to 100 µg/mg of mitochondrial protein) inhibited state 3 and FCCP-uncoupled respiration without significant perturbation of state 4 respiration. However, the inhibition of state 3 was more pronounced than FCCP-uncoupled respiration, reflecting a specific interaction with the phosphorylation system. The transmembrane electric potential ($\Delta\Psi$) of mitochondria after ADP addition also decreased in the presence of both carbon nanoparticles. For the same range of fullerene concentrations (up to 15 µg/mg of mitochondrial protein), the inhibitory effects promoted by C₆₀ on mitochondria function were more pronounced than those induced by C₆₀(OH)₁₈₋₂₂. Altogether, these results demonstrate the importance of the surface chemistry of fullerene nanoparticles in their interactions with prokaryotic and eukaryotic biological systems and, will pave the way for the establishment of structure-activity relationships and new biological applications of carbon nanoparticles.

S. M. Santos is recipient of a fellowship from Fundação para a Ciência e a Tecnologia, Portugal (SFRH/BD/35285/2007).

N°PL4

ENVIRONMENTAL INTERACTIONS

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Nanomaterials (NMs) are expected to be a key in innovation breakthroughs and to lead to many new applications by 2020 (Roco 2011; Roco, et al. 2011b). However it is also well accepted that Nanomaterials will be transferred from the research laboratories to industry and finally to the end-consumer, only if issues on societal challenges such as sustainability and acceptance will be perfectly taken into account.

Behind this issue a key question concerns the risk of nanomaterials and nano-products. Even if a large piece of data is dedicated to address the danger and toxicity of NMs few data exist on the exposure side, the second essential aspect of risk assessment. Environmental exposure will be based on many possible abiotic and biotic processes affecting stability (bio-degradation), fate, transport, and transformation of released nanomaterials. More over as function of the different stages of life cycle of product incorporating NMs, the structure, shape and properties of released NMs will vary.

The talk will detail many possible interactions occurring while NMs are released in the environment that will affect the environment and consumer exposure. The aim of the presentation is to determine the methodology to better constrain the transfer, transformation and ecotoxicity of by-products released from nanoproducts / nanocomposite during their life cycle. It will also be a question of the possibility to propose solution for their remediation.

***Acknowledgment:** The project was supported by the French national programs NANOALTER (INSU/EC2CO/CYTRIX), AGING NANO & TROPH (ANR-08-CESA-001) and the FP7 NEPHH project (CP-FP 228536-2).*

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N°O4a-1

EFFECT OF TiO₂ NANOPARTICLES ON LARVAL DEVELOPMENT OF SWALLOWTAIL WITHIN A FOOD CHAIN

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As uses become more widespread, nanoparticles are inevitably discharged into the environment in increasing concentrations and are potential environmental health hazard. TiO₂ has been said to be less harmful to human health and widely used for cosmetics and food additive.

On point of ecotoxicology, we examined the effects of TiO₂ nanoparticles consisting of both anatase and rutile forms (80/20) on larval development of swallowtail *Atrophaneura alcinous* addressing the transfer from plants to animals. *A. alcinous* eggs were collected on the nettle leaves, *Aristolochia debilis* which is the larval food plant, and the larvae were reared under the same condition as outdoors from May to October in Japan. The stems with several leaves of the plant were exposed in D.W. with 10µg/ml TiO₂ nanoparticles and then the 1st instar larvae fed the leaves for 2weeks until 5th instar larvae. The control larvae were fed the leaves of stems kept in D.W.

Using X-ray analytical microscope (XGT-5000 HORIBA), TiO₂ nanoparticles were detected in the exposed leaves, furthermore, in the gut of the larvae with troubles at molt after fed the leaves and not that of the control larvae. The molt failure was about 60% in the larvae fed TiO₂ exposed leaves. In these results, *A. debilis* was the primary producer to uptake the TiO₂ nanoparticles and the larvae were the primary consumer, which caused detriment the larval molt deathly in *A. alcinous*.

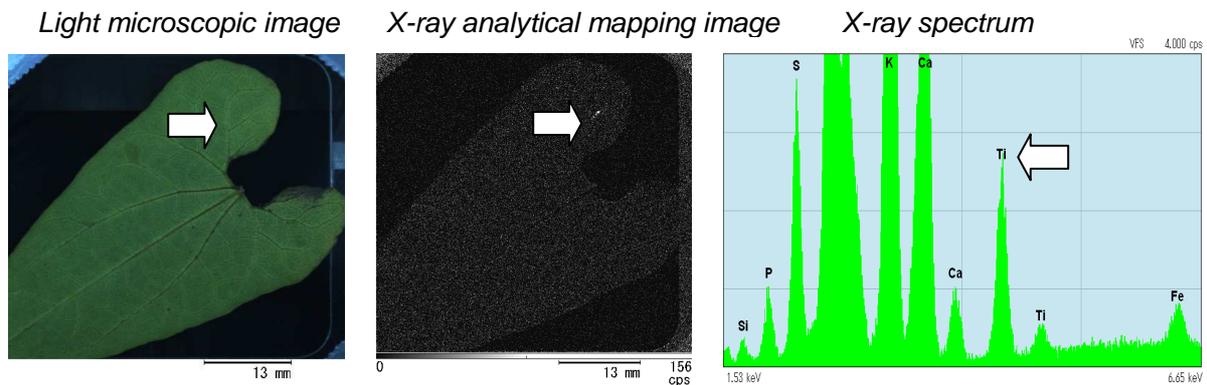


Figure1: Detection of TiO₂ nanoparticles (arrows) in the leaves

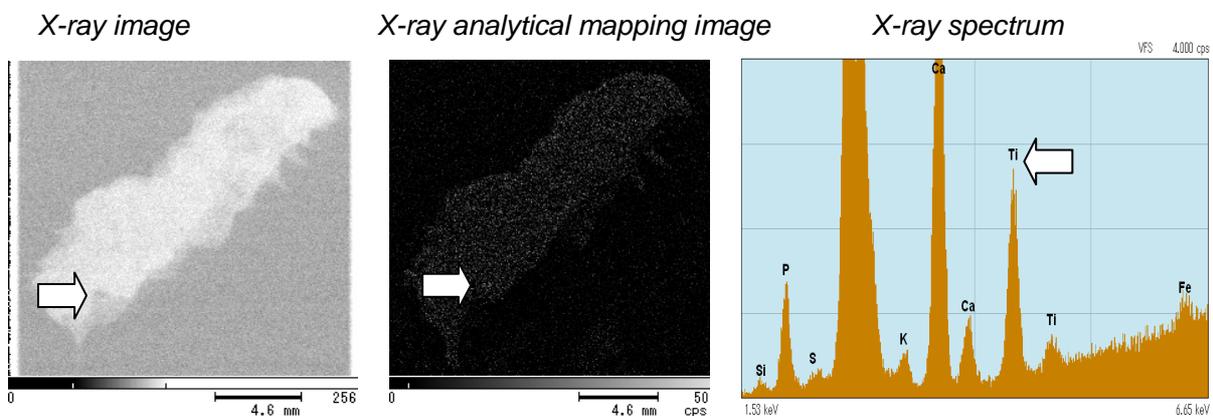


Figure2: Detection of TiO₂ nanoparticles (arrows) in the larva

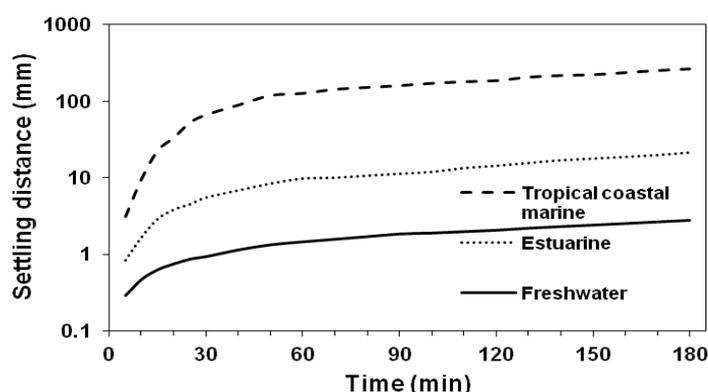
N°O4a-2

**ROLE OF WATER TEMPERATURE IN THE FATE, TRANSPORT, BIOAVAILABILITY OF
ENGINEERED NANOPARTICLES IN AQUATIC ENVIRONMENTS**

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The influence of water temperature on the aggregation and dissolution kinetics of the zinc oxide nanoparticles (ZnO NPs) (mean diameter ~40 nm) was investigated. Samples of 100 mg/L ZnO NPs were incubated at 15, 25 and 35 °C, similar to the surface temperature of freshwater, estuarine, and tropical/sub-tropical coastal marine ecosystems, respectively. The natural organic matter (NOM) content, pH, electrolyte type, and ionic strength (IS), were adjusted on the basis of the water chemistries of typical aqueous systems. Specifically, the time-dependent hydrodynamic diameters (HDDs) and sedimentation plots were obtained over the first 3 h and after 24 h using time-resolved dynamic light scattering (DLS) and UV-visible spectroscopy, respectively. The settling distance was further modeled for the aggregates with various HDDs according to the Stokes' sedimentation equation. The dissolution kinetics was studied over 48 h in terms of the percentage of the released zinc ion. The samples were orbitally shaken to simulate water body circulation in real aqueous media.

The results showed that the HDD increased at elevated temperatures, termed as temperature-induced aggregation, while dissolution was reduced. The aggregation at higher temperatures further hindered the dissolution due to the decrease in the surface area of the NPs. We express this phenomenon as aggregation-suppressed dissolution. The maximum aggregation was reached in the tropical coastal marine environment with the HDD >3 µm, and the released zinc ion of 9.2% was obtained in cold freshwater. Based on the results, the aggregation rate of 0.326 nm.s⁻¹ was estimated for the former within 3 h, and the dissolution rate of 2.97 × 10⁻⁵ mol.L⁻¹.s⁻¹ was calculated for the latter within 12 h, respectively. Finally, genuine samples of the tropical marine and freshwater were examined by spiking them with ZnO NPs, and the results were compared with their synthetic samples (typical NOM of 5 and 30 mg C/L, pH of 9.0 and 7.5, electrolyte type of CaCl₂-NaCl and NaCl, and IS of 100 and 10 mM NaCl, respectively). No significant deviation was observed amongst these samples, suggesting that the modeling data with respect to the synthetic and natural water samples agreed well with one another. The results of this study allow the anticipation of the fate, transport and bioavailability of the ZnO NPs in a variety of aquatic systems, and provide useful data for the environmental risk assessment of the exposure of ZnO NPs to water column and benthic organisms.



N°O4a-3

**MWCNT ACCUMULATION DURING HYDROPONIC EXPOSURE OF WHEAT AND RAPESEED:
QUANTIFICATION AND DISTRIBUTION**

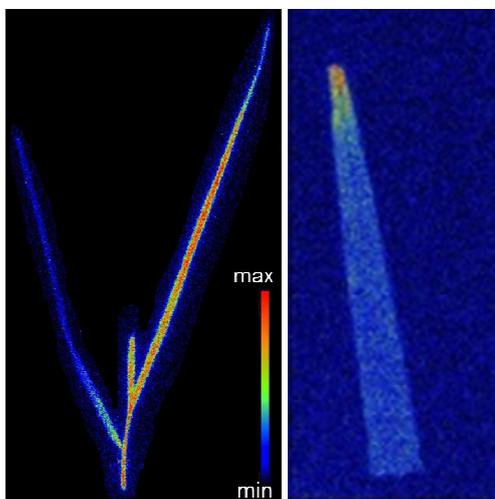
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Contamination of the environment with carbon nanotubes is forecasted to increase in the next years, leading to plant exposure and particularly exposure of agricultural crop. The only quantitative exposure data available to date which can be used for risk assessment come from computer modeling.

The aim of this study was to demonstrate and provide quantitative data relative to MWCNT uptake and distribution in agricultural crops, together with an evaluation of their toxicological impact.

By exposing roots of wheat and rapeseed plantlets to uniformly ¹⁴C-radiolabeled MWCNTs, prepared by coating with gum Arabic or humic acids, we demonstrate their uptake and root-to-shoot translocation in both species. We provide distribution images and quantitative measurements of MWCNT leaf accumulation. Less than 0.005% of the MWCNTs to which plants were exposed were transferred from exposure medium to plant shoot. Contamination with MWCNT did not impact seed germination, root elongation, plant biomass and evapotranspiration, contrary to single-walled carbon nanotubes (SWCNT), which induced higher root elongation. Neither did it induce any modifications in photosynthetic activity or cause oxidative stress in plant leaves. Our results suggest that if environmental contamination occurs, MWCNT transfer to the food chain via food crops would be low.



¹⁴C radioimaging of a wheat plantlet exposed to MW¹⁴CNT

Reference:

C. Larue, M. Pinault, B. Czarny, D. Geogin, D. Jaillard, N. Bendiab, M. Mayne-L'Hermite, F. Taran, V. Dive, M. Carrière. Quantitative evaluation of multi-walled carbon nanotubes uptake in wheat and rapeseed. *Journal of Hazardous Material*, 228: 155-163.

N°O4a-4

BENTHIC FOOD CHAIN STUDIES WITH TiO₂ NANOPARTICLES

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Titanium dioxide (TiO₂) nanoparticles are currently used in a wide variety of consumer and industrial products. As these products are used and disposed of, they may release nanoparticles to the aquatic environment; where their toxicological impact is a topic of growing research interest. To date, most of the research into the effects of TiO₂ nanoparticles in the aquatic environment has focused on pelagic organisms, that is species suspended in the water column and on individual species, not food chains. However, recent reports in the literature show that when TiO₂ nanoparticles enter the aquatic environment, they readily aggregate and sediment from the water column, which may result in benthic organisms being more exposed organisms to TiO₂ nanoparticles than pelagic organisms. Furthermore, nothing is known about how exposure at one level of a food chain may effect higher levels of a food chain.

A simplified benthic food chain consisting of a heterotrophic biofilm and bacterivorous nematodes (*P. acuminatus*), obtained from the same river system, was examined to answer three questions: 1) what effects does direct exposure to TiO₂ nanoparticles may have on the heterotrophic biofilm and nematodes, 2) can TiO₂ nanoparticle be passed from the benthic biofilm to bacteria grazing nematodes, and 3) what effects does exposure through the food chain have on nematodes. To determine the effect of particle characteristics on toxicity and food chain transfer, flame synthesized and solution synthesized TiO₂ nanoparticles with a variety of sizes and surface coatings were employed. Quantification was enabled by: doping the TiO₂ nanoparticle with a low abundant element, using radiolabeled or stable isotope enriched TiO₂ nanoparticles. Toxicological endpoints for heterotrophic biofilm exposure to TiO₂ nanoparticles included measures of biofilm activity (i.e. extracellular enzyme activity or ATP content of the biofilm) and presence or absence of a species as determined by denaturing gradient gel electrophoresis. End points for nematode toxicity included nematode reproduction, nematode growth and nematode ATP content.

First results show that upon exposure to TiO₂ nanoparticles heterotrophic biofilm ATP content decreased for flame synthesized and solution synthesized TiO₂ nanoparticles (figure 1). The extent of the decrease was greater for solution synthesized TiO₂ nanoparticle which are smaller in size (33 nm hydrodynamic diameter) and positively charged (33 mV zeta potential) than the flame synthesized TiO₂ nanoparticle, which have a hydrodynamic diameter of 130 nm and a zeta potential of -11.4 mV. Indicating that smaller particles and particles with a more positive surface charge exhibit greater effect towards the energy content of the biofilm.

When a microcosm consisting of biofilm, nematodes and water was dosed with TiO₂ nanoparticles, 80% of the TiO₂ nanoparticle mass was associated with the biofilm, while less than 0.1% of the mass was associated with the nematode or water fractions. Next experiments will focus on the transfer of TiO₂ nanoparticle from the biofilm to the nematode and further effects of TiO₂ nanoparticles on the biofilm and nematode.

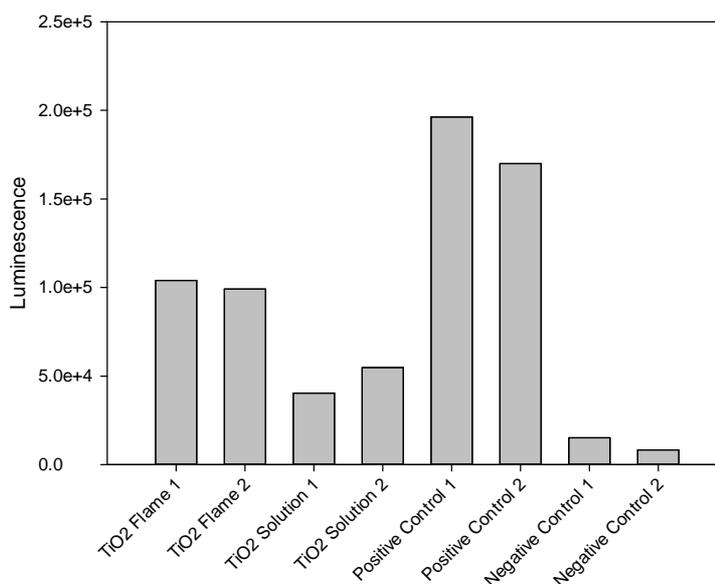


Figure 1) ATP content of heterotrophic biofilm upon exposure to TiO₂ nanoparticles

N°O4a-5

MODELING THE FATE OF NANO-TiO₂ IN THE RHONE RIVER – THE IMPORTANCE OF HETEROAGGREGATION WITH NATURAL COLLOIDS

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Environmental fate models are important tools for deriving predicted environmental concentration (PEC) values in a proactive risk assessment of engineered nanoparticles (ENPs). For this purpose, we need to understand and mathematically describe relevant transport and transformation processes acting on ENPs once they enter natural environments. From the basis of established multimedia environmental fate models for organic pollutants, we developed a new concept of environmental fate modeling for ENPs with process descriptions based on the specific properties of ENPs [1]. Our new fate modeling framework is highly flexible and can be adjusted to different ENPs and various environmental settings.

A key process governing the environmental fate of ENPs in natural waters is the heteroaggregation of ENPs with naturally occurring colloids in the water column. Deposition of ENPs attached to natural colloids determines the distribution of ENPs between water and sediment and the potential of ENPs to be transported with the water flow of a river (Figure 1). Despite the importance of the heteroaggregation on the fate of ENPs in natural surface waters, this process has not been widely studied to date. In this study, values for the attachment efficiency ($\alpha_{\text{het-agg}}$) for the heteroaggregation between TiO₂ nanoparticles (NPs) and natural colloids in samples from the Rhone River were determined by analyzing their aggregation kinetics with laser diffraction. These $\alpha_{\text{het-agg}}$ values were then incorporated in our newly developed river model [1] that was adjusted to the dimensions of the Rhone River. Our model enables the prediction of steady-state concentrations of TiO₂ NPs in the water and sediment compartments along the course of the Rhone River and thereby contributes to an improved exposure assessment of TiO₂ NPs in natural environments.

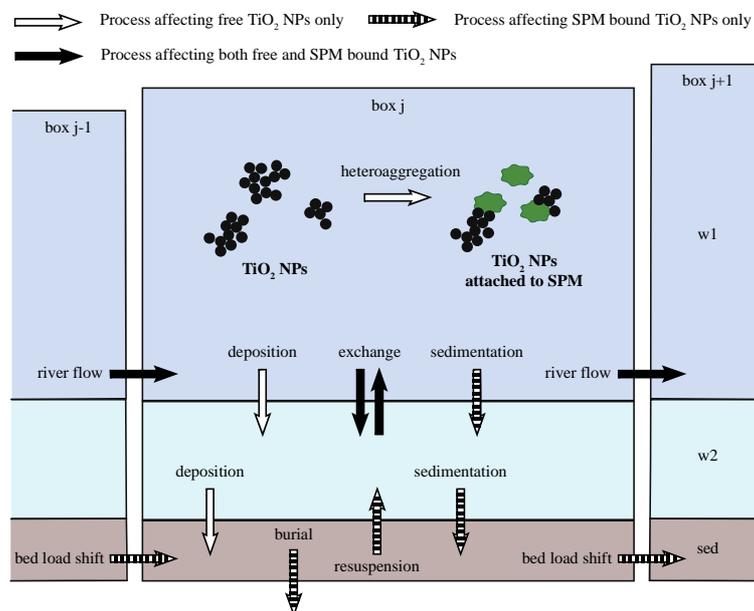


Figure 1: Processes governing the distribution of TiO₂ NPs between the moving water (w1), stagnant water (w2) and sediment (sed) compartments of the Rhone River model

[1] Praetorius, A., Scheringer, M., Hungerbühler, K., Development of Environmental Fate Models for Engineered Nanoparticles – A Case Study of TiO₂ Nanoparticles in the Rhine River, *Environ. Sci. Technol.*, 2012, 46 (12), 6705–6713

N°O4b-1

**AGED TiO₂-BASED NANOMATERIAL USED IN SUNSCREENS: IMPLICATIONS ON
ESCHERICHIA COLI SENSITIZATION TO TOXIC METAL**

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Aging of nanomaterials refers to several processes such as changes in size, coating, shape, surface chemistry and reactivity. Recently, a TiO₂-based nanocomposite, used in sunscreens as UV protectants, was aged in mild conditions, under light and in water, to mimic release of this nanomaterial in the environment. This nanocomposite consists in a TiO₂ rutile core that absorbs UV and a coating made of Al(OH)₃ and polydimethylsiloxane layers to avoid photo-oxidation reactions and the generation of reactive oxygen species (ROS). When dispersed and aged in water under light, a stable colloidal phase (50 to 700 nm) is formed. The organic PDMS is desorbed and oxidized but the Al(OH)₃ stays as confirmed by Al NMR [1], [2].

We have examined the properties of aged-TLite in terms of ROS production and impact on *Escherichia coli* and mutants affected in oxidant and outer membrane stress. We show that aged-TiO₂ based nanocomposite produced neither superoxide nor hydroxyl radicals but released singlet oxygen, in a light intensity dependent way. Aged TiO₂ nanocomposite did not affect the viability of *E. coli* in different nutrient medium in the dark and showed no effect to moderate impact on the survival of cells under low or high intensity UVA. However, we showed that a one-hour exposition of bacteria to aged-TiO₂ nanocomposite (10 mg/L), under low intensity UVA, sensitizes cells to cadmium toxicity. This effect was found to be dependent on UVA intensity and singlet oxygen production.

Singlet oxygen is a selective factor affecting bacterial species dynamics. We conclude that the environmental impact of TiO₂-coated nanocomposites on bacteria could be concerned with oxidative stress, mediated by singlet oxygen, and sensitization of cells to other toxics present in the environment.

[1] Labille (2010) *Environ Pollution* 158, 3482-3489

[2] Auffan (2010) *Environ Sci & Technol* 44, 2689-2694

This work was part of the "AgingNano&Troph" project financially supported by the "CES" programme of the French Agence Nationale de la Recherche (ANR-08-CESA-001-01).

N°O4b-2

CHARACTERIZING NANOPARTICLES REACTIVITY: STRUCTURE-PHOTOCATALYTIC ACTIVITY RELATIONSHIP

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Inorganic Nanoparticles (NPs) are the most widely studied nano-scale objects and they are usually chosen as model nanomaterials due to their physical and chemical *signatures*. A proper characterization (size, shape, chemical composition, structure, surface and reactivity) of NPs is therefore of extremely importance, not only to know the objects we are working with but also to assess the possible risks of their manipulation [1]. However, and even taking into account the current effort on the characterization of NPs, there is still a huge lack of information and of specification. The main reason of the present need is that at the nanoscale objects cannot be considered as unchanged entities.

NPs interact stronger with the environment than those in bulk materials, due to their high surface area related to the volume. Processes such as dissolution, corrosion, degradation, aggregation, Ostwald ripening or adsorption –e.g. protein corona in biological media- become much faster and highly dependent on the environment. In fact, while they may take years when talking about bulk materials, they could need few days at nanoscale. In order to well characterize a nanoparticle the physicochemical properties cannot be defined without specifying in which conditions the NP was (environment) and for how long it was there (evolution) when the measurements were taken. NPs age and do so accordingly to its environment.

Against this backdrop in stabilizing NPs, the reactivity can be considered as a fingerprint of it. A NP is reactive, and its effect is determined by its reactivity, which in turns depends on the NP morphology and the environment where is embedded. One simple and informative approach to the reactivity of a NP is by studying the photocatalytic degradation of an organic compound [2]. In the present work the photocatalytic activity of SiO₂, ZnO, CeO₂ and TiO₂ NPs solutions has been tested and correlated not only with the composition and the structure of the NPs but also with their media and their aging time. The experiments were performed by following the degradation of Rhodamine B dye (RhB) in aqueous solutions under a 365 nm UV Lamp. It is known that RhB has an intensity peak proportional to its concentration at 553 nm and that it can be easily followed using an UV-Vis spectrophotometer. The degradation rate of Rhodamine B in the presence of NPs was measured in different media with proteins, salts and other NPs to mimic the potential environments that a nanoparticle can find in biology. As expected, while SiO₂ and CeO₂ NPs did not show a relevant photocatalytic activity, ZnO and TiO₂ had a high activity, however, different patterns of degradation could be attributed to the composition, morphology (size and shape) and environment (including surface state). The morphological evolution of the NPs during catalytic operation has also been correlated to the observed reactivity. This was also measured after different aging times. Finally, the NPs were characterized by TEM, UV-Vis Spectroscopy, DLS and X-Ray diffraction to supplement the above information.

Thus, in the present work the photocatalytic activity of some NPs has been evaluated as well as how the environment and the evolution of NPs can affect their reactivity.

1. Bastus, N.G., et al., *Reactivity of engineered inorganic nanoparticles and carbon nanostructures in biological media. Nanotoxicology*, 2008. **2**(3): p. 99-112.
2. Beydoun, D., et al., *Role of nanoparticles in photocatalysis. Journal of Nanoparticle Research*, 1999. **1**(4): p. 439-458.

N°O4b-3

TiO₂ NANOPARTICLES IN COSMETIC SUNSCREEN: EFFECT OF AGING ON PHYSICO-CHEMICAL AND CYTOTOXIC PROPERTIES OF BOTH PLAIN FORMULATION AND EXTRACTED NANOPARTICLES

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Nanoparticles are used over large scale in variety of consumer products. In March 2011, the nanotechnology consumer products inventory was containing more than one thousand products, including 33 sunscreens². However, this enthusiasm for nanotechnology is inevitably related to environmental and health concerns. In this regard, hazard and risk assessment remain uncertain with current knowledge: toxicological studies have to be correlated to physico-chemical specifications³. Structure, size, morphology or coating of nanoparticles are crucial to biological activity. Besides, nanoparticles are seldom studied when integrated in a complex physical and chemical matrix such as in a cosmetic formulation⁴.

The objective herein was first to understand the mechanisms of destabilization of a formulation prepared in the presence of nanoparticles, and their connections to potential cytotoxicity. To that purpose, an oil-in-water emulsion, as a typical sunscreen cream, containing different cosmetic grade TiO₂ nanoparticles was developed and their toxicity studied. The impact of two lipophilic coatings and different concentrations of nanoparticles on the emulsion properties was tested. Aged formulations, under classical conditions at room temperature or under accelerated conditions at 50°C, with or without TiO₂, were compared to a freshly prepared one, and extensively characterized by several analysis techniques such as rheology, microscopy, and particle size measurements. Changes in emulsion stability and aggregation state of nanoparticles were followed and studied over time. Stable emulsions, with or without nanoparticles, were obtained as well as destabilized ones, for which destabilization phenomena were identified. These results were associated to cell viability/proliferation testing on human cell lines. Another goal was to characterize nanoparticles after extraction from the formulation media. Time-dependent surface modifications of these nanoparticles were investigated by both zeta potential and infrared measurements, and cytotoxicity tests were performed on these extracted nanoparticles by using bioassays.

² 'Nanotechnology - Project on Emerging Nanotechnologies' : <http://www.nanotechproject.org/>.

³ H. J. Johnston et al., 'Identification of the mechanisms that drive the toxicity of TiO₂ particulates: the contribution of physicochemical characteristics', *Particle and Fibre Toxicology*, 6 (2009), 33.

⁴ C. Botta et al., 'TiO₂-based nanoparticles released in water from commercialized sunscreens in a life-cycle perspective: Structures and quantities', *Environmental Pollution*, (2011), 1543–1550.

N° O4b-4

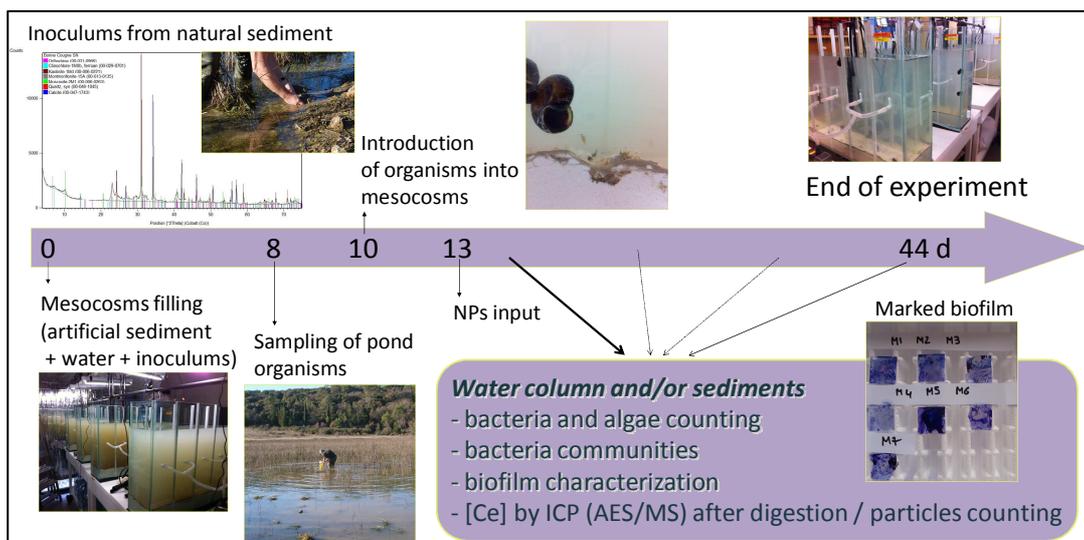
ASSESSMENT OF ENVIRONMENTAL EXPOSURE TO NANOMATERIALS THROUGH MESOCOSMS EXPERIMENTS

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The enhanced physicochemical properties of manufactured (NPs) make them highly attractive for a large range of applications. Among all, ceria (CeO₂) NPs are largely used as oxidation catalysts, gas sensor, polishing materials, but also as UV absorber. Their attractiveness comes from the high oxygen storage capacity related to the easy Ce(III)/Ce(IV) redox cycle associated to the presence of oxygen vacancies. The presence of Ce(III) within the structure of CeO₂ NPs has strong technological and environmental implications. Especially, the reactivity towards living organisms of the CeO₂-NPs will be affected by Ce(III) (prokaryotic and eukaryotic cells).

To date, most of the research done on the environmental risk of NPs only concerns the hazard characterization, and rarely the exposure assessment. Current studies to assess nanoparticles (NPs) safety are based on laboratory toxicology and ecotoxicology approaches in standardized and artificial media, which are not adequate to NPs having unique aspects that not related to the dose. Within the ANR-P2N MESONNET project, this study aimed at understanding the mechanisms of transfer and transformation of CeO₂-based NPs within aquatic mesocosms reaching a realistic ecosystem. Another challenge was to work with low and chronicle doses of NPs contamination, representative of concentrations expected in natural aquatic environments (<1 mg L⁻¹).

During 4 weeks, we have sampled water, sediments and organisms into mesocosms (mimicking a pond ecosystem) to characterize and quantify bacteria and alga communities, [Ce] distribution and speciation, bioaccumulation and corresponding oxidative stress in organisms. Results indicated sediments as preferential accumulation compartment of NPs into mesocosms. Bioaccumulation in pond organisms was also detected (snail, Planorbarius corneus) and correlated with a significant oxidative stress. In addition, using two kinds of CeO₂ Nps for experiments (organic-coated CeO₂-NPs used as paint additives (Nanobyk[®]), and "bare" CeO₂-NPs (Rhodia[®]), we highlighted the influence of NPs surface formulation on their transfer into mesocosms and their reactivity towards organisms.



Mesocosms set-up and sequence for 4 weeks

N° O4b-5

BIOTRANSFORMATION OF CARBON NANOTUBES AND FULLERENES BY HORSERADISH PEROXIDASE

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Within the last two decades there has been a steady increase in the research and production of different forms of carbon-based nanoparticles (CBNs), such as fullerenes and carbon nanotubes (CNTs), for their use in a wide range of applications (e.g. optical, electronic, cosmetic, biomedical). Furthermore, CBNs are also being explored for target delivery in biomedical applications. As a response to the increase in public awareness and concern regarding CBNs in the environment, recent research has focused largely on the uptake, toxicity, and to a lesser extent on modeling the fate of manufactured CBNs in the environment. However, there is scarce information regarding the fate and transport of CBNs, and almost no information regarding the possible biodegradation pathways that CBNs might undergo once released into the human or natural environment. Without this information assessing the possible human and ecological health effects of CBNs remains a challenge for the scientific community. To this end, we are developing analytical methods for the proper characterization, identification, and quantification of selected fullerenes and CNTs and their possible biotransformation products. Here we present the results from an initial set of experiments where different types of CNTs and fullerenes in aqueous suspensions were subjected to enzymatic oxidation with horseradish peroxidase. No substantial differences were observed in SEM images of the CBN suspensions tested before and after the proposed enzymatic degradation. Nevertheless, changes were observed in the agglomerate size, the particulate and dissolved organic carbon concentrations, and the CO₂ concentration in the headspace of the experimental vials during the same treatment. However, this far we are not able to assess the extent of degradation, as the current proposed methodology is not sensitive enough. The use of more sensitive quantitative analysis, e.g. ¹⁴C labeling of CBNs and HPLC-MS of the CBN suspension after degradation, will provide more insightful and more conclusive information.

N°O4b-6

COBALT, TITANIUM DIOXIDE AND NANOSILVER NANOPARTICLES CAUSE SKELETAL DAMAGES IN SEA URCHINS AT PLUTEUS STAGE

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Engineered nanoparticles are increasingly produced to be employed in many commercial products of common use, but their possible toxic effects are not known enough. Preliminary studies report that they can play a role in damaging numerous important biological processes, including skeletogenesis on living organisms. That is very critical in vertebrates, especially during the developmental stages, when the skeleton develops in a genetically programmed way.

The aim of this study is to propose a model of nanotoxicity for aquatic organisms at the developmental stage. The research investigated whether the exposure to different concentrations of cobalt (Co), titanium dioxide (TiO₂) and silver (Ag) nanoparticles can induce skeletal damages in sea urchins (*Paracentrotus lividus*) at the pluteus stage.

P. lividus at pluteus stages were obtained from fertilized eggs exposing sperms at different nanoparticles' concentrations (from 0.0001 up to 1 µg L⁻¹). Non exposed sea urchins' sperms served as controls. The pluteus were made permeable by treating them with glycerol solution and then they were treated in toto with the lectin wheat germ agglutinin (WGA), specific component for binding to the n-acetyl-glucosamine residues. WGA was fluorochrome conjugated, therefore, it was introduced into tissues and visualized through confocal (TCS SP2 Leica, Switzerland) and epifluorescence (Olympus BX60, Japan) microscopic investigations. Fluorescent WGA lectin-binding sites were observed in the mesenchyme cells and in the skeletal rods of nanoparticle-treated pluteus stages. On the contrary, no fluorescence was detected in the controls at pluteus stages. Morphological investigations of the pluteus by means of a Field Emission Gun Environmental Scanning Electron Microscope (FEG-ESEM Quanta 250-FEI, the Netherlands) coupled with an x-ray microprobe of an Energy Dispersion System verified morphological changes and the physical presence of the different nanoparticles in the bodies. No dose-dependent nanoparticle's effects were identified. Morphological anomalies such as the asymmetrical rods of the skeleton and the irregular shape of the pluteus stage were found only in the exposed pluteuses.

In conclusion, the present study suggests that nanoparticles interact with WGA lectin-binding sites in developing mesenchyme and skeleton, causing skeletal alterations and damages. That phenomenon induces malformations in the offspring.

Acknowledgments: The INESE (Impact of Nanoparticles in Environmental Sustainability and Ecotoxicology) project was supported by IIT (Italian Institute of Technology) and POSDRU/89/1.5/S/63663 - Sectorial Operational Programme for Human Resources Development through the project "Transnational network for integrated management of post-doctoral research in Science Communication. Institutional framing (postdoctoral school) and scholarship program (CommScie)".

N°O4b-7

**COMPARISON OF EFFECTS ON CRUSTACEANS: CARBON NANOPARTICLES AND
MOLYBDENUM NANOWIRES**

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Nanomaterial properties may differ from the bulk material as greatly as may their toxic properties. Still, many new materials provide a challenge for the options of toxicity testing. European Environmental Agency within REACH and Environmental Protection Agency within the Framework for Metals Risk Assessment have listed molybdenum in their legislation as a material of (primary-EPA) interest, yet the studies until now have not addressed molybdenum nanoparticle toxicity. Carbon nanomaterials (CNM), in their turn, are one of the nanomaterials with the highest number of potential applications, yet still, little of data exist regarding their fate in environment.

In the current study, *Daphnia magna* standard acute toxicity test (modified EN ISO 6341:1996; freshwater) and *Artemia salina* (ArtoxKit standard method; saltwater) were applied to assess the toxicity of non-modified CNM and molybdenum nanowires.

Carbon black (CB) nanoparticles (Printex XE2 Evonik Degussa GmbH, average diameter of CB particle 30 nm, specific surface area 950 m²/100g, dibutylphtalate absorption 380ml/100g), multi-walled carbon nanotubes (Aldrich, outside diameter 40-60 nm, inside 5-10 nm, length 0.5-500 µm), molybdenum nanowires (Mo₆S₂I₈) and molybdenum nanowires (MoO_{3-x}) were sonicated (with power 1W/ml) in 100 ml *A. salina* and *D. magna* test medium for 5 to 10 minutes to form a stock suspension with total nanoparticle concentration of 10 mg L⁻¹ and diluted for appropriate test concentrations. The monitoring of air temperature, pH and photo-fixation of test organisms was performed. Particle solubility changes in response to varying water salinity were measured, and particle sedimentation was assessed in parallel to the test. Probit regression analysis was used to assess statistically correct results.

Molybdenum nanowires appeared to be partly soluble and the most of the dispersed CNM and molybdenum particles settled after 24 h period. It is believed that due to the low solubility in water and size nanoparticle toxicity did not follow dose-dependence. MoO_{3-x} nanowires turned out to be somewhat more toxic than Mo₆S₂I₈ nanowires, yet more toxic effects have been observed for CNM. *D. magna* showed to be more sensitive to both materials. Further experiments may be needed to explain the toxic effects in interaction with other, including natural, substances.

N°O4c-1

CONCERNS OVER ALUMINIUM OXIDE NANOPARTICLE BASED APPLICATIONS: AN ECOLOGICALLY INSPIRED STUDY USING ENVIRONMENTALLY RELEVANT ISOLATES AND MEDIUM

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Increasing commercial significance of aluminium oxide nanoparticles has raised the ecological concerns. Lack of experimental safety data is apprehensive from a toxicologists' perspective. The focus of the present study is risk assessment and determination of safety end-point along with a meticulous exploration of mechanistic of aluminium oxide nanoparticle induced toxicity in an aquatic environment. This is achieved using a representative set up composed of key components of a natural ecosystem. A short term exposure to ecologically significant algal and bacterial isolates (*Chlorella ellipsoides*, *Bacillus licheniformis*), at environmentally relevant low concentrations (1ppm and less) of aluminium oxide nanoparticle, has been studied using lake water as the medium. The experimental approach was drawn to reveal the physico-chemical behaviour and toxicity end points of nanoparticle in a factual aquatic ecosystem. Observational studies showed a significant toxic response whereas mechanistic probing indicated a surface chemical interaction leading to flocculation and membrane damage along with ion mediated killing of cells. Oxidative stress response was significant at longer intervals which were evidenced by significantly elevated ROS and GSH levels. Fluorescence and confocal microscopy confirmed compromised membrane permeability whereas SEM and TEM provided clear picture of flocculation, surface attachment, membrane damage and morphological distortions. As a whole, aluminium oxide nanoparticles were found induce significant toxic response towards the test species under experimental conditions which might call for a serious thinking in an organised regulation on various applications and disposal methods.

N°O4c-2

**THE ROLE OF SILVER AND VANADIUM TO THE ECOTOXICITY OF SILVER VANADATE
NANOWIRES DECORATED WITH SILVER NANOPARTICLES**

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Recently, a new type of nanowire composed of silver vanadate decorated with silver nanoparticles (10-50 nm) called SVSN-LQES1 with promising antimicrobial activity was described. It is not clear if its toxic effect is related to the release of silver ions and/or vanadium ions or if there are other mechanisms involved. Because this nanomaterial could reach the environment it is important to evaluate its toxicity to aquatic organisms. The objective of this study was to evaluate the contribution of silver and vanadium in the acute toxicity of SVSN-LQES1 to *Daphnia similis* (freshwater microcrustacean). The organisms were exposed during 48h to SVSN-LQES1, AgNO₃ and V₂O₅ solutions, and also the mixture of silver and vanadium at the same proportions present in the SVSN-LQES1. To verify if the silver release were the only cause of the toxicity we performed the test with filtered and non-filtered nanowire solutions. Total silver was determined in the nanowire stock solution using ICP-AES. The percentage of silver released by the nanowires in the solution was 1%. The EC50s of the nanowires solutions non-filtered and filtered were 1.3 and 34 µg.L⁻¹, respectively, indicating that the physical presence of the nanowire increases the toxicity. For silver and vanadium solutions the EC50s were 0.96 and 1400 µg.L⁻¹, respectively. The EC50 obtained for the mixture of silver and vanadium was similar to the solution containing only silver. Therefore vanadium does not seem to contribute to the nanowire toxicity. To further investigate if the toxic effect was related to the release of silver from the nanowires we evaluated the toxicity of SVSN-LQES1 and silver solutions to the estuarine organism *Nitokra* sp. (copepod) at salinity of 20. The obtained EC50s (96h) were 70 µg.L⁻¹ for nanowires, 100 µg.L⁻¹ for silver. In both testing conditions (fresh and salt water) the SVSN-LQES1 was more toxic than the silver solution, although only 1% of the silver present in the nanowire was leached to the media. Therefore only the concentration of silver in the media could not explain the observed toxicity. The toxicity of silver and SVSN-LQES1 solutions were similarly lower in salt media suggesting that Ag ions are involved in the toxicity mechanism of the nanowire because silver ions can react with the chloride from the salt reducing its bioavailability. Considering that the nanowires are trapped by the aquatic organisms, staying the digestive tract of for certain period of time before being eliminated, this behavior would increase the exposure to the silver ions released by the nanowires. In this scenario the concentration of silver ions inside de gut would be greater than the concentration of silver ions in the media. More studies are being conducted to confirm our hypothesis.

Acknowledgements: FAPESP, CNPq, CAPES, FINEP and INOMAT.

N°O4c-3

ADSORPTION OF ORGANIC POLLUTANTS TO AQUEOUS SUSPENSIONS OF CARBON NANOMATERIALS

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Carbon nanomaterials (CNMs) represent a new group of materials with increasing application in new technologies. As a result there is potential for increased releases to the environment. Concerns have risen regarding the possible negative effects CNMs might have on environmental and human health, and several studies have investigated the toxicity of these materials. More recently, the interaction between CNMs and environmentally relevant pollutants has received attention. CNMs exhibit a strong sorption affinity to hydrophobic organic contaminants (HOCs) and may influence their fate, bioavailability, and toxicity. Aqueous ecosystems are likely to be contaminated by nanoparticles, and CNMs have shown to form stable aqueous suspensions by natural processes (e.g. mixing with water and reaction with natural organic matter). Knowledge about the adsorption of HOCs to aqueous suspensions of CNMs is important for determining the environmental impact of both organic contaminants and nanoparticles.

This study examines the adsorption of the PAHs phenanthrene and pyrene, and the estrogenic compound 17 β -estradiol to aqueous suspensions of four types of carbon nanoparticles. The CNMs studied included single-walled carbon nanotubes (SWCNTs) and three multi walled carbon nanotubes (MWCNTs) with differing lengths and outer and inner diameters. The influence of the different physical structures of the CNMs on their sorption capacities to the organic pollutants was investigated. Negligible depletion solid phase microextraction (nd-SPME) was used to sample and concentrate the HOCs from the aqueous phase, and quantification was by gas chromatography mass spectrometry (GC-MS; PAHs) and liquid chromatography mass spectrometry (LC-MS; 17 β -estradiol). This approach permits an accurate determination of the freely dissolved concentration (C_{free}) of HOCs in the CNM-water systems once equilibrium between the aqueous and CNM particulate phase has been achieved. A reduction in C_{free} in a system after addition of CNMs is evidence of adsorption to CNMs, and therefore a corresponding reduction in potential bioavailability to aquatic organisms. nd-SPME extracts only a negligible percentage of the chemical in solution, and does not alter the equilibrium in the sample solution. This is a simple and efficient way of monitoring exposure concentrations of HOCs, also during ecotoxicological experiments.

Examining the adsorption of organic pollutants to different CNMs is the first step in studying the environmental impact of CNMs in aqueous ecosystems. Further work will investigate whether the organic pollutants sorbed to the CNMs are bioavailable to aquatic organisms, and how the CNMs influence the toxicity of the contaminants.

N°PL5

**QUANTITATIVE STUDIES OF PHOTO-INDUCED SURFACE ACCUMULATION AND RELEASE OF
NANOPARTICLES IN POLYMER NANOCOMPOSITES**

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Polymer nanocomposites are increasingly in demand for applications that are subjected to harsh environments. Because polymers are susceptible to degradation by solar radiation, nanofiller in polymer nanocomposites may be accumulated on the composite surface and released into the environments during the composites' life cycle. Such surface accumulation and release potentially pose an environmental health and safety concern. Our previous studies have shown that UV radiation that has wavelength similar to that of the solar spectrum causes photodegradation of the polymer matrix, leading to substantial accumulation of CNTs and nanosilica on the surface of the UV exposed nanocomposites. This study was carried out to quantify the amounts and model the rate of nanosilica release from epoxy nanocomposites as a function of UV radiation doses and exposure temperatures. Specimens of epoxy/nanosilica composites in specially-designed holders were irradiated with a high intensity UV light source. Samples irradiated at different UV doses and at four different temperatures were measured for chemical degradation, mass loss, nanosilica surface accumulation, and nanosilica release. Nanosilica release measurement was accomplished by periodically spraying UV-irradiated specimens with water, collecting runoff water/released particles, and analyzing collected solutions by inductively-coupled plasma optical emission spectroscopy (ICP-OES) using a NIST-developed protocol. Results showed that nanocomposite mass loss, chemical degradation, amount of nanosilica accumulation on nanocomposite surface, and the quantity of nanosilica release increased with UV radiation dose. The experimental release rate is in good agreement with reaction-kinetics model prediction. Effects of temperature on the release rate and activation energy of the photo-induced release will be discussed.

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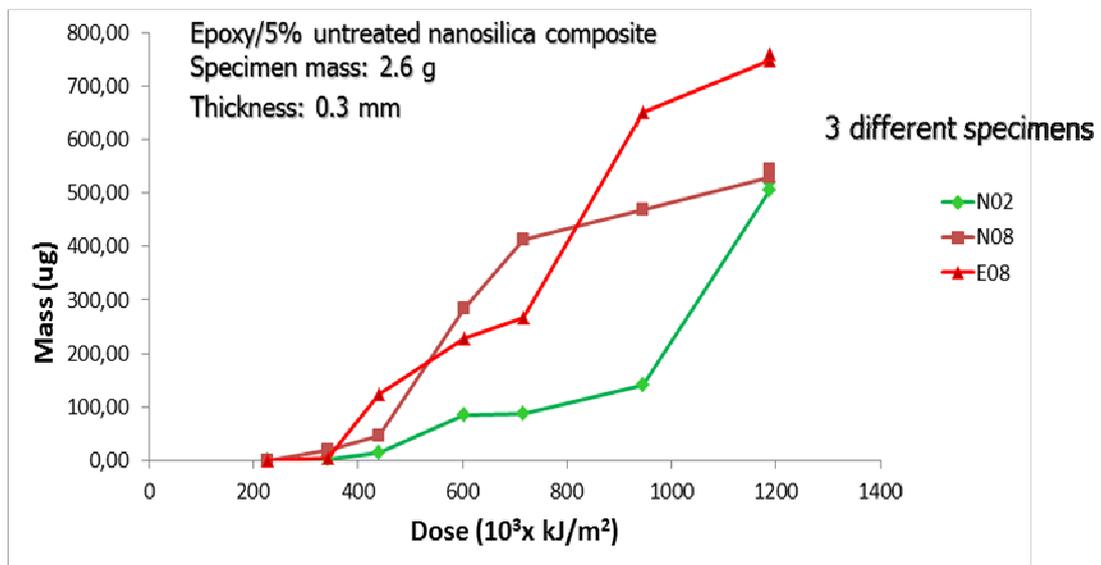


Figure 1. Mass of Si release (measured by ICP-OES) as a function of UV dose.

N°O5a-1

SCENARIOS AND METHODS THAT INDUCE PROTRUDING OR RELEASED CNT'S AFTER DEGRADATION OF COMPOSITE MATERIALS

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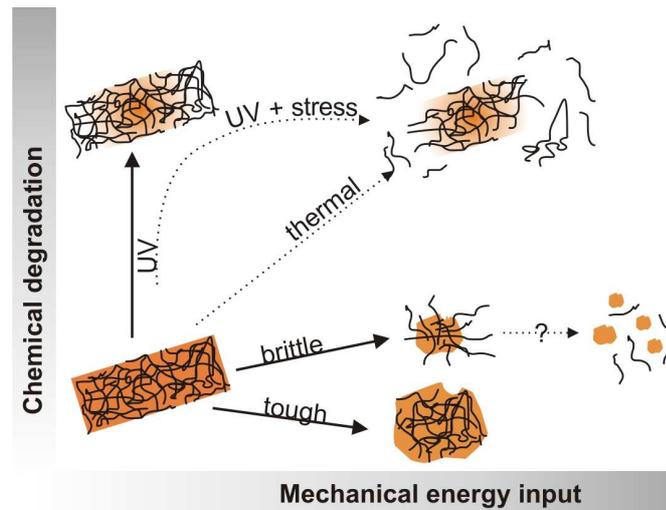
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We hypothesize that release of free CNTs occurs only by a combination of mechanical stress and chemical degradation of the polymer matrix. Nanocomposite materials may be considered a low risk, if the nanofillers remain embedded throughout the life cycle of the products that they are embedded in.



We experimentally address limiting cases:

- Mechanically released fragments may show tubular protrusions on their surface. Here we identify these protrusions on two materials unambiguously as naked CNTs by chemically resolved microscopy and a suitable preparation protocol. Using identical techniques and protocols, protrusions remain absent on certain CNT-polymer composites. Based on classical fiber-composite approaches, we link this phenomenon to matrix materials with only a few percent elongation at break, predicting which materials should still cover their CNT nanofillers after machining.
- By size-selective quantification of fragments we establish as lower limit that at least 95% of the CNTs remain embedded.
- Protruding networks of CNTs remain especially after photochemical degradation of the matrix, and we show that it takes worst case combinations of weathering plus high-shear wear to release free CNTs on the order of mg/m²/year.

Our proposed model, whereof a simplified version is shown in this abstract, allows representing all published nano-release phenomena in a systematic way. Synergy of chemical degradation and mechanical energy input is identified as the priority scenario of CNT release, but its lab simulation by combined methods is far from real-world validation.

N°O5a-2

MONITORING MIGRATION AND TRANSFORMATION OF NANOMATERIALS IN POLYMERIC COMPOSITES DURING CLIMATIC AGING

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The incorporation of small amounts of nanoadditives in polymeric compounds can introduce new mechanical, physical, electrical, magnetic, thermal and/or optical properties. The properties of these advanced materials have enabled new applications in several industrial sectors (electronics, automotive, textile...). In particular, for the nanomaterials (NM) described in this work, MWCNTs and SiO₂ NPs, the following properties have been described: MWCNTs act as nucleating agents in thermoplastics, [1] and change viscosity, affecting dispersion, orientation, and therefore mechanical, thermal, and electrical properties [2]; and SiO₂ NPs act as flame retardant [3] and display improved electrical and mechanical properties.

The work described is focused on the evaluation of the migration and transformation of NM included in polymer nanocomposites during accelerated climatic ageing. To this aim, we generated nanocomposites with different degree of compatibility between NM and polymeric matrix. These nanocomposites were submitted to accelerated aging conditions to simulate outdoor conditions (simulation of the use phase of polymer nanocomposites). The materials studied are PA6 nanocomposites including MWCNTs and SiO₂ NP with different surface properties to influence the compatibility with the polymeric matrix. The generated nanocomposites were evaluated by SEM, TEM, TGA and DSC before and after the aging process, to monitor the compatibility of the NM with the matrix (dispersion of nanomaterial in the matrix, migration during ageing, and polymer properties enhancement).

The dispersion of SiO₂ NPs nanomaterials in the nanocomposites before aging depended on their compatibility with the matrix. However, independently of their compatibility with the matrix, SiO₂ NPs in polymers were aggregated at the end of the aging process. In addition, degradation of the matrix and migration of nanoparticles to the surface was observed in different types of SiO₂ NP aged nanocomposites. The type of MWCNT that was more compatible with the polymeric matrix decreased the degradation of the polymer. Nevertheless, the nanomaterial migrated to the surface during the ageing process.

In order to evaluate the possible changes in the structure of nanomaterials due to the aging process, NM were extracted from the polymer by calcination. The nanomaterials extracted were analyzed by TGA, IR, BET and TEM and compared with calcinated raw NM properties. SiO₂ hydrophilic nanoparticles were not affected by the aging process. However, both types of carbon nanotubes were affected by the aging of the nanocomposites.

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N° O5a-3

RELEASE OF NANOPARTICLES FROM TEXTILES DURING WASHING

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Silver (Ag), both in the nano as well as in other forms, is used in many applications including antimicrobial textiles. Washing of such textiles has already been identified as an important process that results in the release of silver into wastewater. Nano-TiO₂ has the highest production of all nanomaterials and pigment-TiO₂ is a commodity used on the million ton/year scale. Information on the release of TiO₂ from consumer products is therefore an important step in analyzing the potential environmental exposure to TiO₂. This study investigated the release of silver from eight different commercially available silver-textiles and of TiO₂ from six different functional textiles during a washing and rinsing cycle. The released materials were size-fractionated and characterized using electron microscopy.

Three of the textiles contained nanosized silver (labeled or confirmed by manufacturers' information), another used a metallic silver wire and four contained silver in undeclared form. Only four of the investigated textiles leached detectable amounts of silver, of which 34 to 80% was in the form of particles larger than 0.45 µm. Microscopic analysis of the particles released in the washing solutions identified Ti/Si-AgCl nanocomposites, AgCl nanoparticles, large AgCl particles, nano-silver sulfide and metallic nano-Ag, respectively. The nanoparticles were mainly found in highly agglomerated form. The identified nano-textiles showed the highest antimicrobial activity, whereas some of the other textiles, e.g. the one with a silver wire and the one with the lowest silver content, did not reduce the growth of bacteria at all.

Analysis of fiber cross-sections of the sun-protection clothes showed that the TiO₂ was contained in the fiber matrix. Five of the sun-protection clothes released low amounts of TiO₂ (0.01 to 0.06 wt % of total Ti) in one wash cycle. One textile (with antimicrobial functionality) released much higher amounts of Ti (5 mg/l, corresponding to 3.4 wt % of total Ti in one wash cycle). Size fractionation showed that about equal amounts were released as particles below and above 0.45 µm. After 10 washings, only in two textiles significantly lower Ti contents were measured. Electron microscopy showed that the TiO₂ particles released into washing solution had a roundish appearance with primary particle sizes between 60 and 350 nm that formed small aggregates with up to 20 particles.

The results show that different silver textiles release different forms of silver during washing and that among the textiles investigated AgCl was the most frequently observed chemical form in the wastewater. The results also indicate that functional textiles release some TiO₂ particles, but that the amounts are relatively low and mostly not in the nanoparticulate range.

N° O5a-4

RELEASE OF TiO₂ PARTICLES FROM PAINTS CONTAINING PIGMENT TiO₂ AND/OR NANO-TiO₂ BY WEATHERING EXPERIMENTS

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In order to promote a responsible and sustainable development of engineered nanomaterials (ENM) in building industries, it is necessary to study the life cycle of the used ENM and their potential risk for humans and the environment. We therefore investigated the release of TiO₂ particles from paint (pigment-TiO₂ and nano-TiO₂). The paint was applied on fiber cement panels (1950 mm x 750 mm) and exposed in a climate chamber to 113 wetting/drying cycles during one month. Each cycle was a succession of 3 hours of UV light, 0.5h of raining and 2.5h of drying. The drainage water was collected at selected times and the Ti-concentrations and other mineral elements (Si, Ca, Mg...) were quantified by plasma mass spectrometry ICPMS and plasma optical emission spectrometry ICPOES measurements. The bulk analysis revealed that the release of TiO₂ particles was extremely low, representing only 0.02% of the applied TiO₂. No differences were observed between paints containing nano-TiO₂ or not. The SEM-EDX investigations realized on the panels before weathering showed that the TiO₂ particles were well spread and strongly embedded in the binder. SEM images taken after exposure revealed that the TiO₂ particles identified by EDX analysis still well linked to the paint matrix (see Figure 1).

Additional leaching experiments were performed under laboratory conditions in order to better understand the effects of experimental parameters such as dilution factor, medium composition, age of paint, type support material, and UV exposure. These tests were performed in small containers (17 cm x 17 cm x 6 cm) on small panels (10 cm x 10 cm) on fiber cement and plaster. The containers were filled with the appropriate media (deionized water, tap water, rain water) and the panels were shaken for 24 hours at low intensity. The released Ti found by ICPMS showed that the dilution factor, the type of medium, and UV exposure did not influence the quantity of Ti released. A significant increase of the quantity of TiO₂ release was observed for paint applied on plaster support and for aged paint.

Leaching experiments with milled and UV-aged paint were also performed in water with 2 g/l of paint. Under these conditions the nano-TiO₂-containing paint released significantly more particulate Ti than the control paint and about half of it in the nano-range (see Figure 1).

This work showed that depending on the conditions some release of Ti could be observed from the investigated paint. However, under the most natural conditions in the climate chamber almost no release was observed and no difference between the paints with or without nano-TiO₂ was observed.

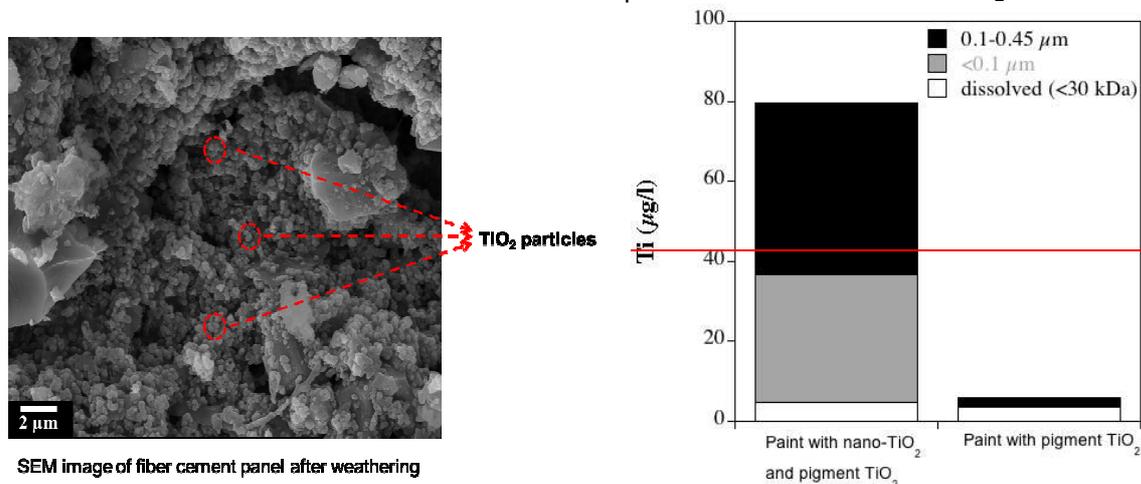


Figure 1: Fiber cement panel after weathering experiment (left side); Size fractionation of Ti release from paints containing pigment and nano-TiO₂ (right side).

N° O5a-5

RELEASE OF CeO₂ NANOPARTICLES UPON AGING OF ACRYLIC WOOD COATING

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With the increasing number of nano-enabled products, the entry of nanomaterials in the environment seems unavoidable. The risks of release are obvious for nanomaterials dispersed in the liquid phase, nano-aerosols, or dry powder, and have therefore focused most concerns until now. On the contrary, nanomaterials embedded in solid matrices have long been considered safe, and their contribution to environmental emissions was largely neglected. Yet, recent studies[1–3] show that important releases may also take place on these products, as a consequence of physical aging. It is then critical to study these emissions more extensively, so that they can be quantified and integrated to risk assessment models.

In the past years, semiconducting nanoparticles (ZnO, TiO₂, CeO₂) have appeared as a good alternative to organic UV-absorbers for paint stabilization. Several products making use of this technology are already available on the market but no data exists on the potential release of nanoparticles to the environment upon aging of the paint.

In this work we studied an n-CeO₂-based paint additive and investigated its aging behaviour when incorporated to an acrylic coating and deposited on wood substrate. Two artificial aging procedures were applied, alternating UV/vis irradiation to simulate sunlight and (i) periodic immersions (batch experiments) or (ii) rain cycles (experiments in climatic chamber) to favour surface wash off and transfer nanomaterials to the liquid phase.

Ce leaching could be evidenced in both cases. The kinetics of emission was established as a function of UV/vis dose and set the basis for comparison between the two procedures. In the search for indicators of nanoparticles release, an evaluation of paint degradation was performed according to ISO standards, and related to cerium emissions. The mechanisms of release were deduced based on combined microscopy and spectroscopy analysis.

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[3] J. Olabarrieta, S. Zorita, I. Peña, N. Rioja, O. Monzón, P. Benguria, L. Scifo, *Appl. Catal. B: Environ.* 123–124 (2012) 182.

N° O5b-1

**RELEASE-ABILITY OF NANO FILLERS FROM DIFFERENT NANOMATERIALS
(TOWARD THE ACCEPTABILITY OF NANOPRODUCT)**

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It is of great interest to set up a reproducible and sensitive method able to qualify nanomaterials before their market introduction in terms of their constitutive nanoparticle release-ability in usage. The objective of developing such measurement techniques is double: optimization of the “hooking” of the nanoparticles in the matrix and perhaps one day, qualifying the nano products before any market introduction.

This paper summer up some methods used to evaluate the release ability of nanofillers contained in paints, cotings and the results obtained.

Within the EU funded FP7 NanoHOUSE project, focused on nano-based paint and coating products for facades, an important experimental work is devoted to the quantification of NP (nanoparticles) release during the use and ageing of indoor and outdoor coatings. Nano-based paints, formulated by the industrial partners involved in the project were applied on suitable panels. Coated panels were then exposed to UV light, weathering and submitted to abrasion tests according to available ISO standard. Release depends of the intensity of the mechanical stress. As could be expected release of free NP from paints matrix in the air for normal use is neglectible. Very low release of micronic and submicronic particles even after hard abrasion and no free NP were detected from some paints. Emission from the weathering exposed material could increase by comparison with the non-weathered material.

In the frame of a collaboration between FP7 NanoHOUSE and FP7 NanoSustain projects abrasion was performed on other nanomaterials: ciments covered with TiO₂ nanoparticles and glass sheets coted with ZnO. For some materials, the emission of the weathering exposed material increase by comparison with the non-weathered material.

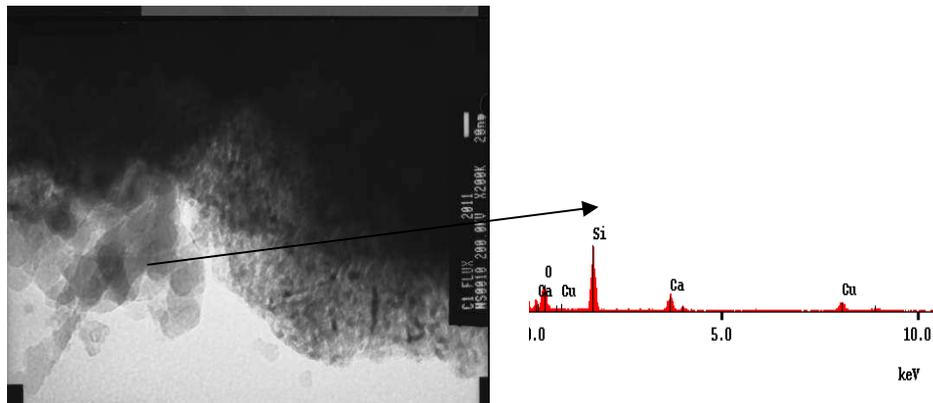


Fig.1 Observation of abraded particles from paint on TEM (Transmission Electron Microscopy) grid

N° O5b-2

NANOMATERIAL RELEASE FROM NANOCOMPOSITES DURING REWORKING PROCESS.

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During the last decade, nanocomposites have developed to be one of the most successful groups of nanotechnological products. New characteristics and/or improvement of mechanical properties have been achieved with ENMs as functional fillers. Therefore, nanocomposites are extremely attractive for several applications as aerospace components, automobiles, energy storage etc. However, these new products could be associated with new risks for human and environmental safety. Detrimental effects have been documented for several ENPs when tested as pure compounds. Recent results suggest that free ENPs are not released from nanocomposites during mechanical wear of nanocomposite products and the toxicities of wear dust are similar to that of conventional composites. However, there is still a great need for detailed studies to assess the potential release of ENMs during specific life-cycle stages of a broader range of nanocomposites. Finishing, renovating and destruction of nanocomposites are activities with high risk of ENP release. Here we present a comprehensive study of the airborne particulate matter generated by mechanical degradation of several composite materials with and without ENP. The studies included sanding paint/TiO₂ or epoxy/carbon nanotube (CNTs) nanocomposite surfaces, breaking glass/zinc oxide nanocomposite sheets with a hammer or cutting and tearing nanocellulose-based paper. The experiments were performed in a closed aerosol chamber with simultaneous measurement with CPC, FMPS, ELPI and APS to describe the evolution in particle number concentrations and size-distributions. Filter samples were collected for characterization by SEM (Fig. 1). Some structural and morphological differences were observed depending on the type of nanocomposites. The source strength, S_i (cm⁻³s⁻¹), was found to be size dependent and gives information about the rate of particles generated in a continuous process. Comparison between the S_i parameters derived from sanding different materials makes it possible to identify potential effects of ENP addition to a composite. For example, it was found that S_i for 263 to 384 nm-size particles is larger for CNTs epoxy-based nanocomposites than for the reference material.

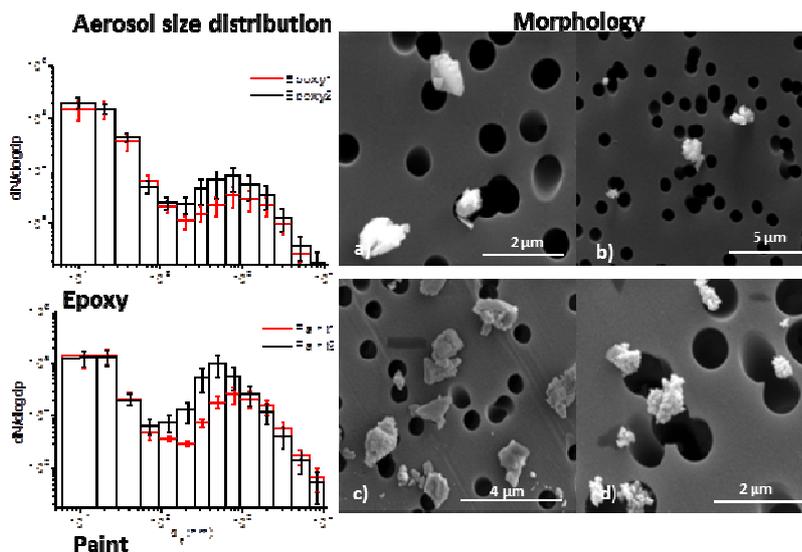


Figure 1 ELPI aerosol size distributions and scanning electron microscopy images of dust collected on a PC filter during sanding Epoxy-based composite and Paint with nanofillers b) Epoxy2 and d) Paint2 and without nanofillers a) Epoxy1 and c) Paint1.

N° O5b-3

NANOPARTICLE RELEASE FROM NANOCOMPOSITES DUE TO MECHANICAL TREATMENT AT TWO STAGES OF THE LIFE-CYCLE

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The ability and the quantity of nanoparticle release in the air from engineered nano-composites due to certain treatment processes can be determined systematically by suitable exposure studies in laboratory for estimating potential risk to health, safety and environment (Kuhlbusch et al., 2011).

Nano-composites are subjected during their life-cycle to different treatment processes, where a denuding of embedded nanoparticles can occur by matrix material destruction due to external impacts like sunlight, moisture or temperature fluctuations. In turn, this is probably associated with a higher release risk as shown by Kaegi et al. (2008) for the transition of TiO₂ into the aquatic environment.

In order to characterize the nanoparticle release due to mechanical treatment on nano-composite samples in different states of life, a project was established in cooperation with the Verband der Mineralfarbenindustrie e.V. (VdMi), Germany that was supported by the German Federal Ministry of Economics and Technology (BMWi) under grant 01FS10004.

The analysis method of Göhler et al. (2010) was adapted to characterize the (nano)-particle release due to wind erosion, dynamic friction and sanding from artificially weathered nano-composites (coatings and polypropylene composites) with pigments of different sizes. Experimental setups with suitable aerosol measurement devices were designed to determine particle size distributions and particle concentrations, while SEM-, TEM- and EDX-Analyses were performed for particle material identification. With attention to appropriate process parameters, more than thousand analyses were performed and measurement data were evaluated according to Göhler et al. (2010) and ISO TS 12025:2012.

Sanding-based results show that the weathering of the coatings led to an increase of the nanoparticle release in comparison to the non-weathered samples, whereas a decrease due to the weathering was observed for the polypropylene composites. The addition of nano-pigments to the matrix material results in some cases to a decrease in the particle release, for both non-weathered and weathered coatings. Some coatings based on non-nanoscaled pigments show a higher release of nanoparticles in comparison to the non-doped ones.

The authors will show how the process simulations were realized and how the measurement data were evaluated for the quantification of the particle release. In addition to the results of particle release, the possibility to determine emission factors for the comparison of different treatment processes and their return in real scenarios will be discussed.

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Kuhlbusch TAJ, Asbach C, Fissan H, Göhler D, Stintz M. Nanoparticle exposure at nanotechnology workplaces: A review. *Part. Fibre Toxicol.*, 2011, 8:22.

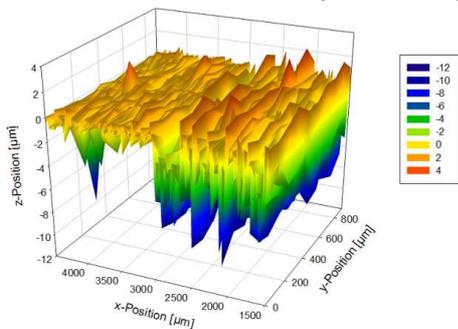


Figure 2. Changes in sample surface roughness due to sanding.

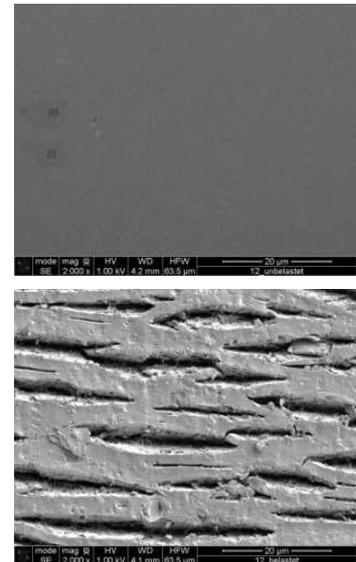


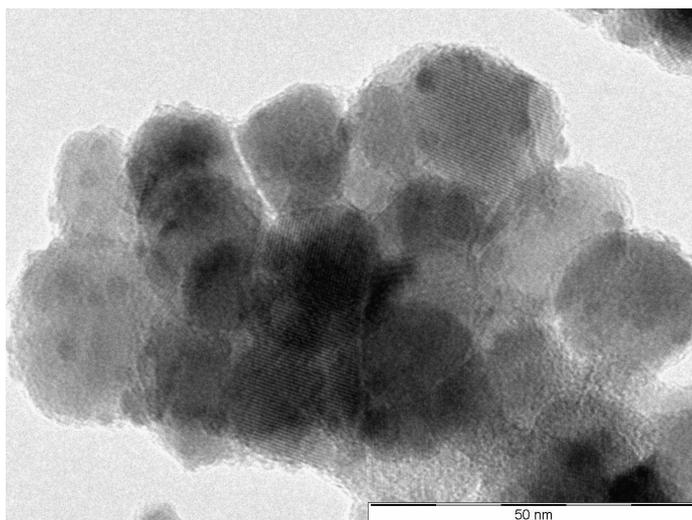
Figure 1. Example of changes on the sample surface due to artificial weathering.

N°O5b-4

ON CHARACTER OF COARSE, FINE AND ULTRAFINE PARTICLES IN AUTOMOTIVE BRAKE WEAR DEBRIS

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Over one billion registered vehicles worldwide generate considerable amounts of pollutants. While the emissions related to exhaust gasses and tire wear were addressed extensively, the released brake wear debris and its impact were studied to a considerably lesser extent. Our previous work indicated that the released wear debris can have negative impact on environment. A characteristic automotive brake pad is a multicomponent composite typically formulated of more than 10 constituents. Manufacturers of brake pads worldwide use several thousand different raw materials, e.g. various metals and their compounds, carbon-based components and many others. This contribution addresses the character of wear debris released from a model “low metallic” friction material, used in a typical brake in Europe, USA and Asia. Brake samples were subjected to the standardized brake dynamometer test simulations (Link Engineering) and collected wear particles were further studied using a High Resolution Transmission Electron Microscopy (HRTEM – Hitachi 7100) equipped with the Energy Dispersive X-ray Microanalysis (EDX –Noran Voyager), and used in a series of genotoxicity bioassays (Ames test and SOS Chromotest) and immunofluorescence detection of human DNA damage. Our experiments demonstrated that airborne wear particles with sizes between 10 nm and 20 µm were released into the air (see the Figure). Elemental and phase analysis revealed the presence of numerous compounds which were not present in the original brake material. Nano-sized Cu, Fe, and Sn oxides and carbon particles were confirmed in the released coarse, fine and ultrafine wear debris fractions. The genotoxicity tests demonstrated mutagenic potency of wear particles in all bioassays used. These findings proved contribution of non-combustion processes associated with braking of automobiles to nano-particulate air pollution which may potentially pose health risks in areas with heavy traffic.



TEM image of ultrafine particles emitted from a model friction composite

The study was supported by the projects MSMT ME10121, CZ.1.07/2.3.00/20.0074, SP2012/45 and SP2012/65

N°O5b-5

ABRASION TESTS ON MWCNT COMPOSITES: INFLUENCE OF CNT DISPERSION STATE AND FILLER/MATRIX INTERFACE

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Carbon nanotubes (CNT) reinforced polymer-based composites represent a significant opportunity in terms of mechanical reinforcement and electrical and thermal conductivity improvements. However specific issues for nanotubes and related composites on human health are still under studied. It is strongly expected that standards and regulations on carbon nanotubes and on carbon nanotubes composites should appear soon. Due to their high aspect ratio they could migrate into breathing apparatus (because of their small diameter) and remain stuck to the walls (due to their length) causing damages like pulmonary fibrosis or cancer.

Exposition of human people to carbon nanotubes must be controlled and an occupational exposure limit must be defined. That is why suppliers of carbon nanotubes have large interest to predict rules in controlling carbon nanotube release during the use of materials prepared from nanotubes, especially under abrasion or other cyclic mechanical solicitations. A key point is to check if a good dispersion state is a required condition to decrease isolated carbon nanotubes release.

The different works which focused on the carbon nanotubes/polymer matrix interface in nanocomposites show that the interfacial interactions between a nanotube and the polymer are supposed to be stronger than ones between two nanotubes (in aggregates). The main aim of this study is to confirm this hypothesis and to check if carbon nanotube dispersion state has an influence on the potential release of isolated nanotubes in the nanocomposite.

To tailor and to characterize the CNT dispersion state in the polymer matrix different high shear dispersion tools were used to improve the dispersion state as much as possible and electronic microscopy (TEM) was used to well identify if carbon nanotubes are isolated, stacked in bundles or in aggregates. Two parameters were defined from image analysis in order to quantify this dispersion state : dispersion degree and micron-size aggregates percentage.

As the dispersion state of carbon nanotubes in polymer-based nanocomposites was known and controlled, standardized abrasion tests were performed in a glove box in order to simulate the wear use of a nanocomposite during its lifecycle, i.e. to generate particles. Three different polymers were chosen as matrix of nanocomposites: two thermoset epoxies and a thermoplastic polyamide PA11. For each type of polymer, the neat polymer matrix, a nanocomposite with a good dispersion state and another with carbon nanotubes as micron-size aggregates were processed. Controlled parameters were defined to calculate and to analyze the applied shear stress in order to be compared with interfacial shear stress between two nanotubes and at nanotube/polymer interface. The abrasion was simulated with two specific tools in order to simulate two different uses : sanding and scratch.

Released particles were collected on TEM grids and by particle sizing devices and these ones were analyzed in term of number, size and nature thanks to different characterization methods. Granulometric data, TEM micrographs and EDX measurements were all performed and founded to be influenced by several parameters amongst which the carbon nanotubes dispersion state.

Carbon nanotubes were found in the abraded particles but never isolated from other polymer particles but could be linked to released polymer particles via Van der Waals interactions and physical entanglement. It clearly appears that the dispersion state of CNT has an influence on the shape and the aspect of released particles.

For more details and results (submitted publications) :

-Influence of CNT dispersion state on filler/matrix interface during breakage in MWNT epoxy nanocomposites

- Probing and characterization of nanoparticles release from polymer filled with MWNT by the simulation of two wear processes on three nanocomposites - Influence of the dispersion state on the CNT release from polymer by sanding and scratching.

N° O5b-6

CHARACTERIZATION OF RELEASED PARTICLES DURING ABRASION TEST OF NANO-CHARGED CONSTRUCTION MATERIALS

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The development of nanomaterials offers opportunities for innovation (ex: concrete containing nanomaterials) in the field of construction and public works [1]. However, these nanomaterials have a potential effect on humans and the environment [2]. Therefore, it is necessary to study its possible release under different conditions of use, in order to, if necessary, change the design of the product. The study presented here focuses on the potential emission of particles in air with the use of nano-structured titanium dioxide (TiO₂). The nano-structured TiO₂ exhibits photo-catalytic properties which render a product with the advantages like reduction in atmospheric pollution and auto cleaning of surfaces.

The approach adopted is a "maximizing" approach. The surfaces considered are subjected to abrasion. Operating points having highest emissivity were selected from data obtained by varying the operating parameters – normal mass, abrasion speed and duration of the test. The experimental set up [3] consists of an emission chamber, a Taber type tribometer, real time aerosol measuring devices (CNC, APS, SMPS), and a system for collecting the sample particles for their further microscopic analysis using TEM [4-6]. Two types of products have been considered: the surface of a mortar simulating the surface of a conventional concrete and the surface of a dry mortar developed by compression simulating the surface of a pavement (construction materials using cement and sand).

The results show that, for some operating points, aerosol can be generated during the abrasion of these two products. The particles produced were mainly in micronic size range. No free nano-particles were observed. Elements from the nanomaterial were observed in micron sized particles, the composition of which is representative of the original matrix (mortar or dry mortar equivalent respectively to conventional concrete and a paving surface).

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N° O5b-7

ON NANOPARTICLES RELEASE FROM POLYMER NANOCOMPOSITES FOR APPLICATIONS IN LIGHTWEIGHT AUTOMOTIVE COMPONENTS

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he nanomaterials provide a new avenue of progress into technological development especially in automotive industry where lightweight components are on high demand. By manipulating materials on the very basic atomic and molecular levels the property of a given material can be specifically altered to suit the purpose of intended applications. However, nanomaterials (nanoparticles and nanolayers) have a more complex nature in physicochemical properties and surface reactivity than their larger counterparts. Therefore, the release of these nanomaterials as dust during crushing or drilling may lead to serious health hazards for humans and the surrounding environment. This study, supported by the NEPHH (Nanomaterial-related Environmental Pollution Health Hazards Throughout Their Life Cycle), addresses a key important question about polymer nanocomposites on whether nanomaterials can be released from physical process of nanoproducts.

In particular, nano and micro reinforced crash cones were manufactured throughout a two-step extrusion process and final injection moulding of the nanogranulates. Secondary fillers consisted of nanoclays (DELLITE® 43B and 72T, Laviosa) and nanosilica (AEROSIL® R 974 and 200, Degussa, Evonik Industries), and as micro filler glass spears with a diameter between 20-50 µm were introduced. Size-resolved particulate emissions from various silica based composites during low velocity impacts were evaluated. Physical characterization of the number concentration and size distribution of sub-micron particles from 5.6 to 512nm was carried out, for the different composites. The investigation observed that nano and ultrafine airborne particles were emitted from all nano and microsilica filled composites. The effect of the matrix selection and nanofiller content in the polymer composites will be presented and conclusions drawn.

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ABRASION BEHAVIOR OF AN EPOXY-BASED NANOCOMPOSITE WITH RAW- AND FUNCTIONALIZED CARBON NANOTUBES

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Epoxy/carbon nanotubes (CNTs) nanocomposites exhibit excellent mechanical properties. Compared to the pure epoxy, they have additional properties like electrical conductivity and thermal conductivity. They are used for applications where low weight, high strength, and high conductivity are required. The potential hazard of such materials has become a significant concern to researchers, manufacturing industries and customers. The exact environmental and health impact of nanoparticles released into the ambient either during manufacturing or in applications is still unknown and a matter of debate.

Recently, we have shown that free standing CNTs are released from epoxy nanocomposites when an abrasion process is applied (Schlagenhauf et al. 2012). For this study the release of raw and functionalized CNTs from an epoxy/CNT nanocomposite was investigated. The hypothesis that functionalized CNTs show a different behavior is tested, since it is known that by functionalization, the interaction between epoxy and nanofiller is improved (Gojny et al. 2003) and it is also possible to establish covalent bonds between the CNTs and the epoxy resin (Shen et al. 2007).

The raw CNTs were purchased from a mass production company and the functionalization as well as the characterization of the CNTs was carried out in our laboratory. The raw CNTs were functionalized either by carboxylic groups or by ethylenediamine. The CNTs were incorporated into the epoxy resin by ultra-sonication and by three roll milling (Hollertz et al. 2011).

An experimental set-up has been established to perform abrasion, particle measurement, and collection all in one. The abrasion process was simulated with a Taber Abraser. The particle size distribution of the abraded particles was measured in the size range from 10 nm to 20 µm by a scanning mobility particle sizer (SMPS) and an aerodynamic particle sizer (APS). Additionally, collected particles were investigated by scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and transmission electron microscopy (TEM).

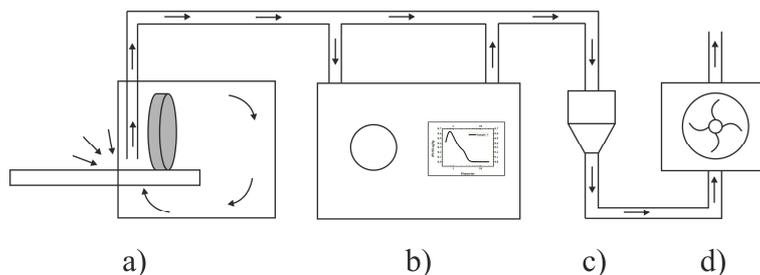


Fig. 1 Schematic of the experimental setup, consisting of (a) the abrasion area covered by an enclosure chamber, (b) the particle-analyzing device, (c) a particle collection device for image characterization, and (d) a pump. The air flow directions are indicated by arrows.

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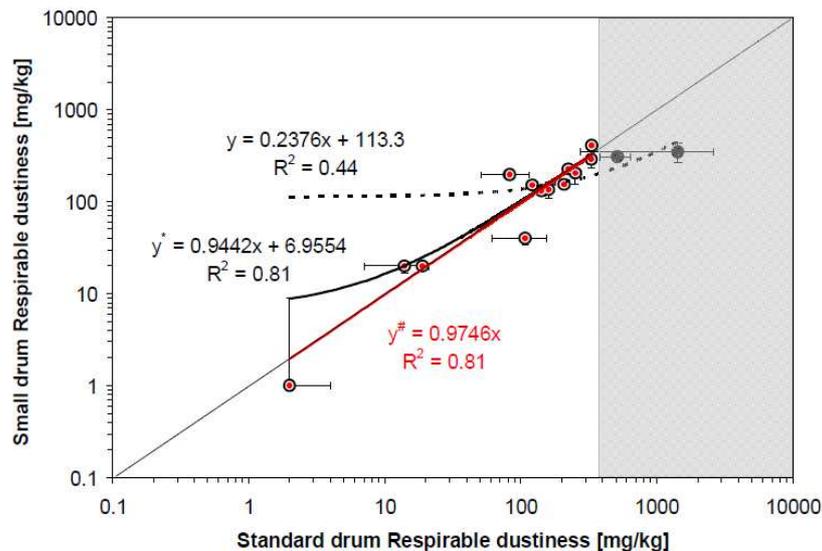
N° O5c-1

NANOMATERIAL DUSTINESS - A COMPARISON BETWEEN THREE METHODS

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Dustiness testing is a method to generate data for assessment of the potential exposure during handling of nanomaterial powders. EN15051 prescribes two test methods using either a rotating drum or a continuous drop method. Recently, the rotating drum was miniaturized to enable safer testing and using less nanomaterials (2-6 g vs. a fixed 35 cm³ in the standard drum). One alternative is the Vortex shaker method that enables testing of only 0.5 ml. In the EU FP7 project NANODEVICE and the Joint Action project NANOGENOTOX, we have compared the dustiness data using the standard (LRD) and the small rotating drum (SRD) and the Vortex shaker system (Vortex).

Ranges in SRD respirable dustiness varied from below the detection limit to more than 3000 mg/kg and there was excellent agreement between the dustiness levels obtained by the SRD and the LRD systems ($R^2 = 0.44$ to 0.81 ; $p \leq 0.002$ see Figure). Dustiness levels obtained by the Vortex shaker and the SRD are also in general agreement of the ranking ($R^2 = 0.51$ to 0.66 ; $p \leq 0.052$ depending on regression model), but the Vortex shaker dustiness levels are considerably higher due to shaking the material for 60 min as compared to 1 min agitation in the RD systems.



*Respirable dustiness index generated by the miniaturized (Small) plotted against the standard (Standard) rotating drum. Broken line is linear regression using all samples; * regression analysis omitting the two highest values ; # regression forced through (0,0) and omitting the two highest values.*

Comparing the Vortex/SRD ratio vs. the SRD respirable dustiness levels also shows that the relative dustiness levels of from the Vortex system has a tendency to increases with decreasing dustiness levels as compared to the values from the SRD.

Comparing the dustiness levels with physicochemical characteristics of the powders show no significant linear correlations. However, for a series of pure and functionalized calcites showed linear correlation ($R^2 = 0.97$) with specific surface area of the powders.

We conclude that dustiness of nanopowders vary greatly and the mechanism of agitation (dropping vs. shaking), but the relative ranking is generally kept. Dustiness can not be predicted from powder properties and appropriate tests methods are required to assess the potential exposure from powder handling.

N°O5c-2

**NANORELEASE – DEVELOPING METHODS TO MEASURE RELEASE OF NANOMATERIALS
FROM SOLID CONSUMER PRODUCTS**

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Nanotechnology is a promising technology that enables the creation of new materials and devices at the nano-scale for use in medicine, energy, electronics, cosmetics, packaging, and many other fields. Governments and industries around the globe are investing billions into research to understand the possibility and limits of these newer technologies. Nanomaterials have found applications in a wide variety of consumer products. Scientific and public concerns about the potential impacts of nanomaterials on human health and the environment have been raised. A fundamental question is “Are nanomaterials released from consumer products?”

The NanoRelease project is intended to support the development of methods to understand the release of nanomaterials from consumer products, and foster the safe development of nanomaterials. The project is a multinational public and private partnership with administrative support by the ILSI Research Foundation. The project consists of three phases:

- (1) Phase 1 – Nanomaterial selection (completed): specific criteria were used to rank nanomaterials; the first workshop in May 2011 in Washington DC informed the steering committee on which nanomaterial to choose; multi-walled carbon nanotubes (MWCNTs) in polymer have been chosen for the first evaluation.
- (2) Phase 2 – Methods evaluation (in progress): three task groups were formed to compile and catalogue database of data/information and to develop white papers; the second workshop took place in June 2012 in Bethesda in which three task groups presented white papers: 1) TG1 – measurement methods: how can MWCNTs release from polymers be measured? 2) TG2 – materials: how do MWCNTs in polymers behave/interact with regards to measurement of release; 3) TG3 – exposure/release scenarios: what are the most likely release scenarios of MWCNTs in polymers? Based on the TG white papers, a final “state of the science” document will be developed.
- (3) Phase 3 – Inter-laboratory testing (2013): the inter-laboratory testing group is currently recruiting laboratories and beginning to design a final workplan for laboratory testing. With a “round-robin” or a similar approach, multi-labs will participate in testing methods with highest potential using a reference MWCNT/polymer system and positive controls for the study of MWCNTs released from selected polymers.

The long-term objective of the project is to specify research needs, fulfill identified research gaps and eventually aim for standardization.

N°O5c-3

**NANOMATERIAL RELEASE FROM PRODUCTS IS NOT RELATED TO HAZARD DATA:
METHODS AND DATA LACKING**

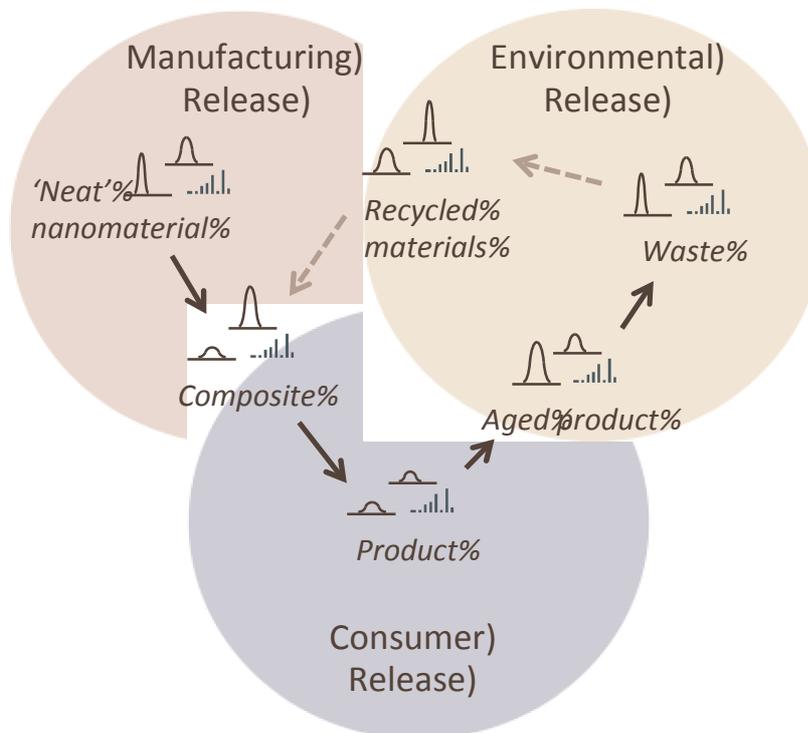
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Advances in incorporation of engineered nanomaterials (ENM) into composites have occurred in tandem with the identification of possible hazards of the pristine ENM prior to compositing. However, review of research of release of ENM from nano-composites conducted for the NanoRelease project (www.ilsf.org/ResearchFoundation/Pages/NanoRelease1.aspx) shows little attention is being paid to ENM release from the composites. Consequently, it is not clear whether potential risks of that material are informed by current ENM hazard research.

A crucial finding of the review is that our understanding of the risks associated with uses of nanomaterials will continue to be insufficient until substantial progress is made on methods to measure ENM release from uses of ENM. In the available reports, consumer products containing nano-silver, nano-titania and carbon nanotubes have been investigated under several exposure scenarios, including, weathering, abrasion, UV and washing.

The data show three possible release scenarios: nanoscale particles of some kind (almost always, similar to materials without added ENM), nano-scale particles with some carrier matrix and some ENM (often), and finally, ENM alone (rare) throughout product life cycle, see Figure 1. This review of the nanomaterial release literature illustrates a data gap relevant to the field of environmental risk assessment. Perhaps most critical, our review suggests that fate and transport modeling, exposure assessment, and risk assessment frameworks in general for ENM uses should not be based on pristine engineered nanoparticles, but rather should be based on the released composites of ENM and the carrier matrix.

Figure 1: Released materials appear to be influenced by the mixture of mixtures in the composite and the release media throughout an article's life cycle.



N° O5c-4

CHARACTERIZATION AND QUANTIFICATION OF NANOPARTICLE RELEASE FROM COMMERCIAL AVAILABLE SPRAY PRODUCTS CONTAINING ENGINEERED NANOPARTICLES.

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Nanomaterials exhibit rich physical and chemical phenomena, and their fascinating and unusual properties have opened up a myriad of applications in various fields. Hence, a tremendous increase in the use of engineered nanoparticles (ENP) in consumer products has been arisen during the last years. In spite of the superior properties, ENP also raise concerns about adverse effects on biological systems and the human health. Especially spray products containing ENP deserve closer attention with respect to the very critical exposure pathway via the lungs. Until now, only a few studies concerning release of ENP from spray products into the environment are available and many questions are still open. To fill this gap, different spray scenarios are investigated with the goal to understand the behavior and fate of the nanoparticles during the spray processes.

The characterization of nanoparticles is challenging. Therefore, we developed different analytical methods to determine quantity, morphology, size and elemental distribution for ENP in spray products and the generated aerosols. For the investigation of the spray dispersion asymmetric field flow fractionation (A4F) coupled to ICPMS was used. This method allows a detailed characterization of particle size and size distributions, concentration and chemical composition of the dispersions.

The determination of the airborne nanoparticles is performed in a glove box setup to ensure reliable spray experiments in a particle minimized environment. In a first step the primary aerosol formation is investigated in detail, in a second phase, the interaction with other aerosols present in the ambient environment should follow. The particle size distribution in the upper size range (>300 nm) is determined by an optical counter (OPC), whereas the lower size range (<500 nm) is measured using a scanning mobility particle sizer (SMPS) (3 min/size scan). Alternatively to SMPS, a fast mobility particle sizer (FMPS) (1 s / size scan) is used. In parallel an electrostatic sampler is employed which allows a sampling of single particles on transmission electron microscopy (TEM) grids for subsequent electron microscopy analysis to achieve information on size (via image analysis), morphology and chemistry of the nanoparticles and agglomerated in the generated spray aerosols.

In a first step, different spray scenarios with commercially available "nano" products were investigated. A special focus was set on distance and spray direction dependency. A model which describes the chances during the spray process should be established based on the findings. Moreover, the data should be used to feed an exposure model to assess the potential uptake via lungs, skin and intestinal tract.

N° O5c-5

Behavior of nanoparticles during high temperature treatment (incineration type)

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In a global context of reduction and follow-up of the atmospheric particle emissions, limiting the release of nanoparticles (NPs) when submitted to waste incineration is a non negligible element. This subject is growing while the implementation of end of life solutions under control are put in place, as a necessary continuation of the increase in the production and use of engineered NPs.

Nowadays waste containing nanomaterials are most of the time treated by elevated temperature (from 850°C to 1100°C) like classical waste in incineration plants. There is no much information about the behavior of NPs during waste incineration to establish which fraction stays in the slag and which percentage becomes airborne and is released outside the plant.

Measuring and especially understanding NPs release submitted to high temperature processes is one of the topics of the NanoSafety Platform at CEA Grenoble.

The first step of this fundamental study has been to develop a specific bench test including a laboratory furnace connected at its exhaust to NPs analysis tools.

Critical parameters such as the mode of introduction of the sample in the furnace, the gas flow adjustments, the sampling point ... have been optimized to ensure good sensitivity and to lead to a reproducible methodology of detection of NPs released during the incineration process.

Preliminary results show that release of NPs from an initial nanostructured sample is significant and measurable with our devices (figure 1). Correlations between NPs emission profile with temperature, size distribution during release and SEM observation were established. Moreover, links between the NP initial properties and their emissivity during heating were examined.

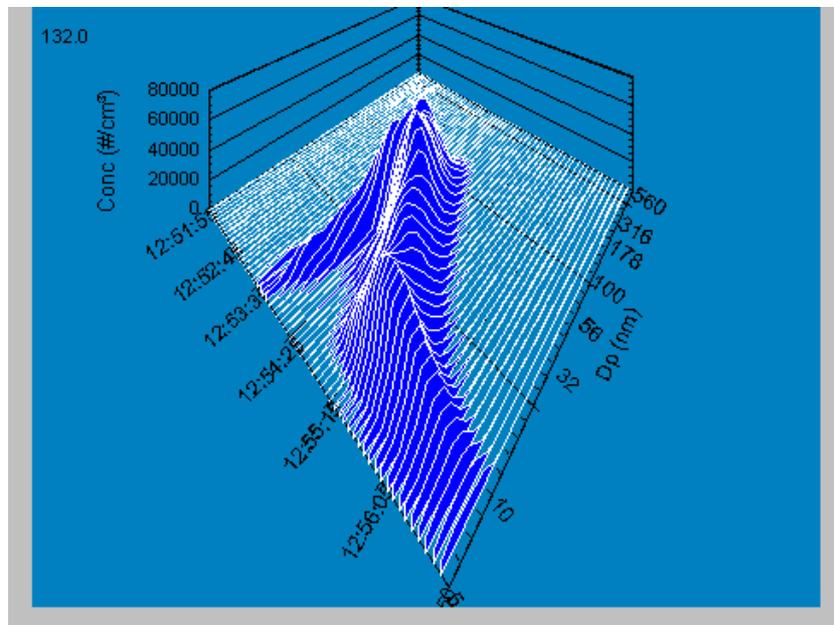


Figure 1: Size distribution of NPs released when heating silver NPs at 1100°C

N°O5c-6

FATE OF CNT-EPOXY COMPOSITE DURING INCINERATION

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Today carbon nanotubes (CNT) are used in increasing amounts in many applications to improve the performance e.g. to increase the strength of the material or to improve the electrical conductivity. CNT enforced epoxy-composites are used in e.g. wind turbine blades and sporting equipment such as ski poles and sticks. At the end of life these CNT-epoxy composites often end up in waste incinerators to generate heat and electric energy as they cannot be recycled in an easy manner. This might generate a release of CNTs to the air or CNTs might remain in the bottom ash.

CNT-epoxy composite chips were incinerated in a laboratory scale incinerator to evaluate the behaviour of CNTs during this process. The composite was co-fired with woodchips and characterisation of the particles emitting were done using aerosol instrumentation such as CPC, SMPS and ELPI. Samples were also collected for electron microscopy analysis and various gas phase species, such as CO₂, CO and O₂, were monitored using FTIR. Combustion experiments were carried out with various amounts of CNT-composite (0 – 20 w%) at good as well as at poor combustion conditions. Also the bottom ash was analysed using various techniques in order to see if the CNTs had combusted or still existed in the ash.

At lower CNT-composite feed, no CNT-structures were found in the fly ash nor in the bottom ash. However, at 20 w% feed the amount of CNT-composite was too high for this type of incinerator, and the CNT composite in the fuel mixture more or less melted together to form largish lumps. Thus CNT structures were found from the bottom ash.

Acknowledgment: The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement 24789 (NanoSustain). Danys Naumenko and Valentinas Snitka from Kaunas University of Technology are acknowledge for the Raman analysis.

N°O5c-7

**RELEASE OF TiO₂ NANOPARTICLES FROM CEMENT DURING THEIR LIFE CYCLE:
STEP OF USE**

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According to the annual report of CEMBUREAU, the world production of cement in 2011 was 3.6 billion tons. A part of this production concerns a new type of cement, called self-cleaning cement which maintains wall fronts clean and white. This property comes from the incorporation of photocatalytic titanium dioxide nanoparticles (TiO₂ NPs) in the cement matrix. During continuous UV radiations exposition, the TiO₂ NPs contribute to accelerate the oxidation (i.e. degradation) of compounds adsorbed at the cement surface. This recent nanomaterial (nanoparticles-based material) is promising as it exhibits improved properties but its environmental validation (in terms of impacts and risk associated with the incorporation of TiO₂ NPs) is also required.

Cement is submitted to alteration when exposed to water (e.g. rain draining on cement wall). An altered layer is then formed at its surface where numerous and complex reactions (cement phase congruent or incongruent dissolution, secondary phase formation, etc.) occur. This layer shows an increased porosity. Cement leaching behavior, and associated element release into the environment, is well described in the literature but the behavior of the incorporated TiO₂ NPs is currently unknown. Release of TiO₂ NPs is suspected, more precisely the emission of nanomaterial degradation residues (NDR) into the environment (waters, soils ...). In this study hypothesis of the TiO₂ NPs release controlled by the surface layer porosity is proposed.

This study focuses to the leaching behavior of cement incorporating TiO₂ NPs and more precisely, to the influence of the porosity on release process. Samples are industrial cements with TiO₂ NPs that were hydrated at the lab-scale in various conditions (addition of 30, 40 or 50 % of water), and so exhibiting different porosity. Porosity was assessed by mercury porosimetry and SEM image analysis. To simulate the alteration phase, static leaching tests (liquid/solid ratio (L/S) of 100) were performed during 7 days. The samples were placed within a dialysis membrane (10 kDa) filled with ultrapure water and immersed in a leachate solution (ultrapure water) to isolate the released particulate fraction from the released soluble fraction. The elements release (particulate and soluble fractions) and its kinetic were quantified by ICP-AES; the altered layer was characterized by SEM and micro-XRF imaging and the speciation of Ti in the particulate fraction (TiO₂ NPs aggregated, still embedded in cement matrix or free) was identified by TEM.

As assumed, no soluble Ti was observed in the leachate. But Ti was detected in the particulate fractions. The release kinetic curves of TiO₂ NPs can be described in three regions: (i) during the first 24 hours, TiO₂ NPs are retained in the cement, (ii) between 1 to 4 days, the porosity does not influence the TiO₂ NPs release with remains constant around 10 ng Ti/g cement/h, (iii) in the last step, the TiO₂ NPs release shows two opposite behaviors depending on the initial cement porosity. It significantly increases to 32.5 and 59 ng Ti/g cement/h for cements hydrated with 30 and 40% of water, respectively. This behavior can be related to the increased cement porosity during leaching. And the TiO₂ NPs release decreases to 2 ng Ti/g cement/h for cement hydrated with 50% of water, i.e. with the highest porosity. The hypothesis of the precipitation of a secondary phase (calcite) blocking the pore space in the altered layer is proposed.

N°O5c-8

**EXPLORING RELEASE AND RECOVERY OF NANOPARTICLES FROM POLYMER
NANOCOMPOSITES USING COMMERCIAL POLYAMIDE-BASED NANOCOMPOSITES AS A
MODEL**

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Much concern has been risen about the risks associated with the broad use of polymers containing nanomaterials. Much is known about degradation and ageing of polymers and nanoparticles and other nanomaterials independently, but very few studies have been done in order to understand degradation of polymeric nanocomposites containing nanomaterials and the fate of these nanomaterials, which may occur in suffering many processes such as migration, release and physicochemical modifications.

Throughout the UE funded FP7 project NANOPOLYTOX studies on the migration, release and decrease or loss of mechanical properties of commercial nanocomposites due to ageing and weathering have been performed along with studies on the feasibility of recovery and recycling of the nanomaterials.

A part of the project includes the uses as model nanocomposites of Polyamide (PA) polymeric matrix containing a 3% in mass of a set of selected broadly used nanomaterials; from inorganic oxides nanoparticles (SiO₂, TiO₂ and ZnO) to multi-walled carbon nanotubes (MWCNT) and Nanoclays . These model nanocomposites were then treated under accelerated ageing conditions in climate chambers and characterization techniques were used to determine the degree of degradation of the whole nanocomposite and possible processes of migration, release and modification of the nanofillers. Obtained data includes studies on the most common mechanical parameters, TEM and SEM imaging of microtomo cuts of the composite before and after ageing, to determine migration and modification of the nanomaterials and ICP-MS data of the ageing waters combined with Thermogravimetric (TGA) studies to determine release of both polymer and nanomaterials throughout the ageing and weathering process.

Additionally, a process to recover the nanomaterials from the polymeric matrix was developed, based on dissolution of Polyamide with formic acid and consecutive steps of centrifugation and washing; with the aim of both recycle those materials for further uses and to study the modification of the nanomaterials due to ageing of the nanocomposite.

N°PL6

DEVELOPMENTS IN NANO PROTECTION

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Nanotechnologies are emerging today all over the world: the exposure risks around workplaces are already present and expected to grow exponentially during the next years¹. Today the toxicity of nanoparticles is a complex matter: there are many different types of nanoparticles and properties that can play a role. In the context of the precautionary principle, protective equipments efficient against nanoparticles are needed.

The question today is: how efficient are the protection equipment's used for other type of risks (Eg. EPI individual protection equipments: filters, protective clothing, gloves) for nanoaerosols and nano colloides? How efficient are the current detection technology to verify the non-exposure of the worker around the workplace? ... Towards a biomarker of exposure to engineered nanoparticles?

This paper attempts to make a point at this moment on this topic by making a literature review and presenting results on this topic at CEA-Grenoble.

¹*Mihail C.Rocco, B.Harthorn, D.Guston, P.Saphira Innovative and responsible governance of nanotechnology for societal development, J Nanopart Res (2011) 13:3557-3590*

N° O6a-1

TOWARDS LARGE SCALE ALIGNED CARBON NANOTUBE COMPOSITES: AN INDUSTRIAL SAFE-BY-DESIGN AND SUSTAINABLE APPROACH

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Industrial production capacity of dispersed carbon nanotube powder has boomed since 2005 [1], mainly conditioned in masterbatches. The coming of industrial production of Vertically Aligned Carbon Nanotubes carpets/arrays/forests (VACNT) is still at its first stages but represents the second generation of safer carbon nanotube production. From an industrial point of view, the main challenges are the control of carbon nanotube characteristics and structure through a safe and low cost process applicable to large surfaces. In this context, Catalytic Chemical Vapour Deposition (CCVD) appears to be a versatile process with great potential. Especially, the aerosol-assisted CCVD process developed at CEA [2], which offers a fast [3] and continuous growth of VACNT directly on various substrates [3, 4] is operated simultaneously from liquid hydrocarbons and catalyst precursors (ferrocene) in a single step.

We present the main results obtained within the framework of the French program NaWaa4, which aimed at demonstrating the feasibility of high surface (> A4 format size) industrial fabrication of composites embedding VACNT in organic matrices. The process of growing VACNT exhibits several advantages regarding safety issues: integrating de facto a safe collecting procedure on the substrate, avoiding additional preparation steps and simplifying handling and protection by impregnation into a matrix. The following steps of the overall process: VACNT carpet functionalization, alignment control and impregnation, can be processed on-line in a closed and safe continuous process and lead to dramatically reduce direct nanotube exposure for employees and users. We collected sample and residues all along the process and found only large composite particles (of size > 100 nm) made of nanotubes into polymer, which are less toxic than volatile CNT powders. This project opens the route to a continuous, roll-to-roll, safer, cost-effective and green industrial process to manufacture composites with controlled and aligned greener « black » carbon nanotubes.

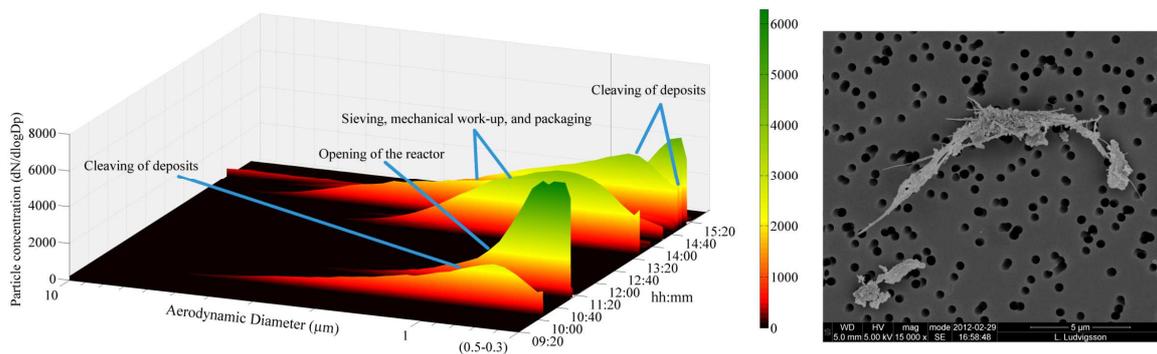
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N° O6a-2

**DETECTION AND IDENTIFICATION OF AIRBORNE MULTI-WALLED CARBON NANOTUBES IN
ARC DISCHARGE PRODUCTION**

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The research, development and production of carbon nanotubes (CNTs) are all rapidly growing, as are the fields of applications. As we know that the fibre structure and properties (such as stiffness and biopersistence) of CNTs can be similar to those of asbestos, and hence potentially hazardous, it is of importance to develop measurement methods to identify and quantify emission sources and personal exposures during production and handling. In this study measurements of emissions and personal exposures were made at a small-scale facility producing multi-walled carbon nanotubes (MWCNTs) using the arc discharge method. This production method is the least studied from an emission and exposure point of view and since several parts of the production takes place without enclosure or exhaust ventilation there are potential exposures. The aims of the study were to (1) examine if emissions of CNTs occur during arc discharge production, (2) describe and classify the types of CNT containing particles that are emitted, their content, shape and origin, (3) examine the differences in emitted particle concentrations and size distributions between production activities, and (4) present a filter/electron microscopy technique as a complement to online instruments. Number concentrations and size distributions were measured, and filter samples collected, in the emission zone (< 10 cm from emission point) as well as in the background (>3 m from the closest identifiable source). The workers carried personal monitoring instruments in the breathing zone. The filter samples were systematically analysed by scanning electron microscopy (SEM) and length–width relationships were determined. By using a ratio condition (3:1) as definition of a fibre, number concentrations of fibres were determined. Further, concentrations of “free” CNT fibres and CNT containing particles were manually determined from the SEM images. Several production activities resulted in sharp emission peaks in number concentration with activity dependent size distributions. Sieving, mechanical work-up and packaging emitted 4 ± 0.6 free CNTs and 11 ± 0.9 CNT containing particles/cm³. SEM analysis of filters in the breathing zone showed concentrations of up to 0.3 free CNT fibres/cm³, three times higher than the 0.1 fibres/cm³ threshold limit value for asbestos fibres.



Number size distribution time series as measured with aerodynamic particle sizer (APS) (left) and a typical CNT containing particle analysed by scanning electron microscopy (SEM) (right).

N° O6a-3

**PLASMA POLYMERIZED COATING AS A PROTECTIVE LAYER OF CARBON NANOTUBES
GRAFTED ON CARBON FIBERS**

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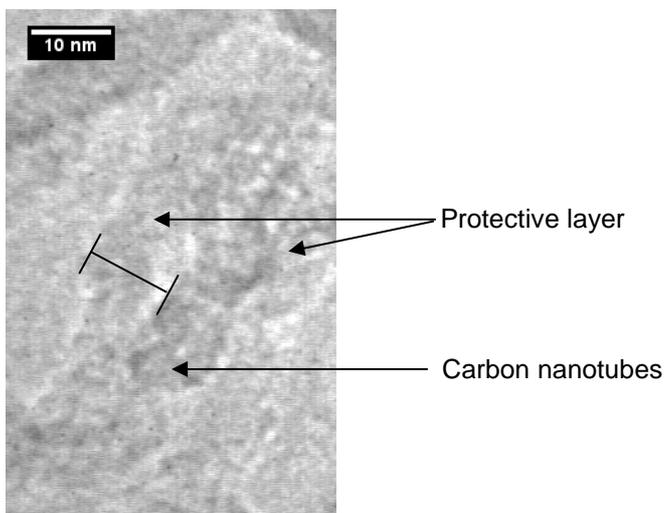
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Carbon fiber reinforced composites are particularly used in extreme conditions, as in aerospace, rather than conventional materials. Indeed they are characterized by higher tensile properties and electrical energy dissipation as well as light weight structure. One of the key points of their properties is the interface between the matrix and the fibers. Thus CNT are grafted by Chemical Vapor Deposition (CVD) on carbon fiber as reinforcement and electrical nanoparticles. Both tows and woven fabrics made of this hybrid fiber are added to thermoplastic or thermoset matrix for composite processing. However handling hybrid fibers may induce unwilling health risk of CNT release. Therefore a protective layer is required both for safety purpose and interface properties preservation.

The coating is deposited by low pressure plasma polymerization of organic monomer. In this pilot, uncoated CNT are confined in the vessel under vacuum instead of atmosphere. Moreover monomer is in gaseous state leading to homogeneous polymer on CNT in addition to ungrafted sites of fiber, as shown by MEB micrograph. The thin protective layer, i.e. about 10 nm as designated by TEM micrograph, does not modify the morphology and the organization of CNT on carbon fiber surface. The chemical composition of the deposited layer is very similar to the monomer and its fragmented chemistry forms as indicated by XPS.

Protective role of coating on CNT grafted fiber is investigated by the way of released CNT counting. Friction constraint is applied on hybrid fibers reproducing drastic conditions textile and composite processes. Furthermore influence of layer on mechanical and electrical properties of fibers is studied. Mechanical properties strongly depend on interfacial properties, and are characterized by means of fragmentation tests. Polymeric coating improves the cohesion between hybrid fiber and matrix. Similarly it reveals correlation between plasma conditions and layer interface into the composite. Conductivity of carbon fiber is enhanced by CNT grafting. The coated layer has not significant effect on surface and volume resistivity of hybrid fibers.



TEM micrograph of CNT coated by protecting organic layer

N° O6a-4

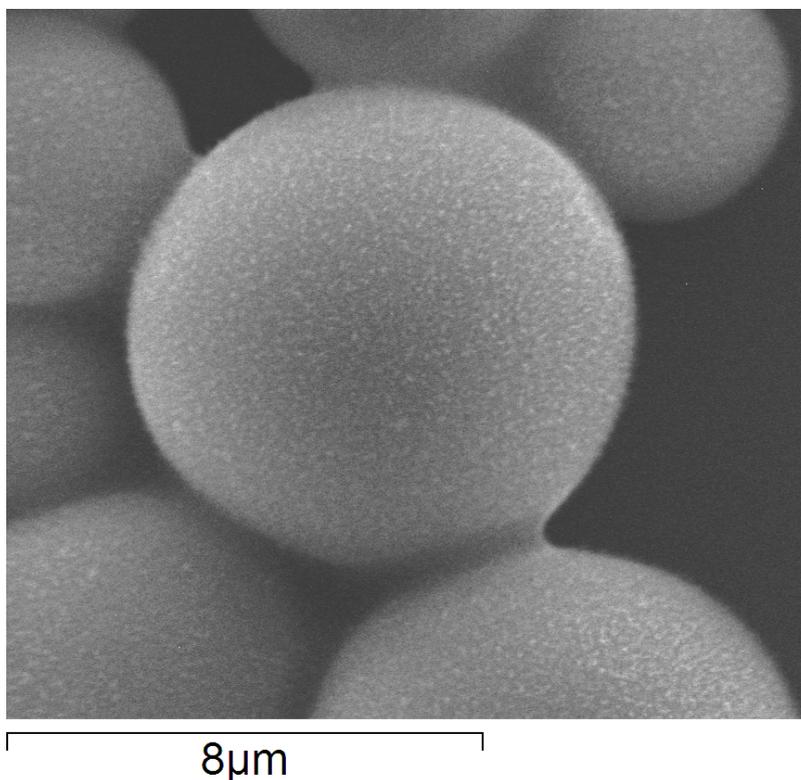
**SILICA-COATING AS PROTECTIVE SHELL FOR THE RISK MANAGEMENT OF
NANOPARTICLES**

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Nanoparticles (NPs) surface functionalization through the application of organic or inorganic coatings allows creating new materials (hybrids, core-shell structures) with engineered properties. In particular, coatings based on silica (SiO_2) have attracted high attention due to their hydrophilicity, biocompatibility, chemical and thermal stability even in aqueous media. The present work is addressed to the production and characterization of SiO_2 -coatings on titanium dioxide (TiO_2) and silver (Ag) NPs dispersed in aqueous solutions (commercial nanosols) with the aim to manage the potential risk that such nanoparticles may generate within an occupational exposure scenario. Two different approaches were followed: i) a colloidal approach, based on principles of heterocoagulation, in which opposite charged NPs of TiO_2 (or Ag) and SiO_2 are forced to coagulate, with a hierarchical structure imposed by their relative size and weight ratio; ii) a chemical approach that consists in nucleation of silica phase on TiO_2 or Ag nanoparticles seeds by starting from silica precursor solutions. In order to increase the adhesion of silica on TiO_2 and Ag surfaces, heterocoagulated sols were spray-dried and subsequently redispersed in water to check the feasibility of such approach at industrial level from the point of view of the properties of the sols. Physicochemical properties such as zeta potential, electrical conductivity, particle size distribution, specific surface area and morphology of the samples produced with different $\text{SiO}_2 : \text{TiO}_2$ and $\text{SiO}_2 : \text{Ag}$ weight ratios were collected and compared. Information about the state of the systems, suitable operative condition for a best control of the processes and indirect evidences of the silica coating were obtained. Finally, phenomena that can predict toxicity of uncoated or SiO_2 -coated TiO_2 or Ag NPs were evaluated and compared in order to check the efficiency of these new engineered nanoparticles.



SEM image of heterocoagulated TiO_2 - SiO_2 sol (SiO_2 : TiO_2 weight ratio = 5:1) after spray-drying.

N° O6a-5

PILOT-SCALE PLATFORM FOR NANOPOWDER SYNTHESIS BY LASER/PLASMA HYBRID PROCESSES

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In the growing field of nanosciences for energy, communication, environment or defence applications, the need for reliable large scale production of tailored nanoparticles is increasing exponentially. Moreover, for health and environment issues, the question of the processes safety takes a peculiar importance. For these reasons, the development of efficient and secured synthesis platforms is addressed worldwide.

Gas phase synthesis processes present several advantages for pilot-scale production of nanoparticles: continuous flow production, high purity and narrow size distribution for obtained particles, large variety of materials, relevant control of particles characteristics (size, composition), production monitoring by real-time in-situ characterization techniques... Among these processes, laser pyrolysis (LP) and inductively coupled plasma (ICP) nanopowders synthesis combine most of these advantages.

In this context, CEA, University of Orleans and Cilas company decided to develop a pilot-scale platform enabling the production of innovative nanomaterials in sufficient quantity (several kg/day) to prepare demonstration samples for industrial evaluation. The Nanosynthese platform uses both LP and ICP processes coupled in a same production unit (same precursors distribution, reactor and powder collection systems). In addition, the possibility to use gaseous, liquid and/or solid precursors and the availability of these two processes ensure the successful synthesis of almost any kind of materials (metals or ceramics, oxide or non oxide). Moreover, the specific design of the reactors offers the possibility to obtain advanced structures such as spheroidized particles or core-shell systems. The purification of scarce materials can also be addressed by this system.

Monitoring of the process will be performed through the development of different in situ real-time characterization processes (LIBS, RF-plasma size measurements, light scattering...) enabling the study of the particles (size, composition, presence of by-products) during their production. Ex situ characterization processes (dynamic light scattering...) dedicated to nanomaterials will also be developed on the platform.

Safety issues will be addressed following two approaches in order to avoid any release of nanomaterials in the lab or in the environment: on the one hand specific working protocols (pressurized rooms, filtered and treated process and lab exhausts, specific personal safety equipment for maintenance phases...) coupled to working environment control by continuous analysis of atmosphere particle content, and on the second hand advanced process integration from the precursors to the products handling and storage (free standing nanopowders liquid conditioning, granulation processes, in-situ direct or coupled deposition for nanocomposite thin or thick layers elaboration).

The Nanosynthese platform will be located in Orleans, R&D programs will start in January 2013.

Acknowledgments: Equipment funded by FEDER, Région Centre, Conseil Général du Loiret, Ville et Agglomération d'Orléans.

N° O6a-6

IN SITU SYNTHESIS OF ANTI-BACTERIAL NANOCOMPOSITE COATINGS BY NANOSAFE-BY-DESIGN SPUTTERING PROCESS

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Anti bacterial surfaces can be obtained by depositing either a photocatalytic material (e.g. TiO₂) or noble metals such as Cu or Ag. In the former case, UV irradiation is required to allow bacteria destruction whereas noble metals present an intrinsic anti bacterial effect. Recent trends consist in associating both photocatalyst and noble metals in nanostructured cermet coatings that consists in a nano dispersion of noble metal in a titanium dioxide matrix.

Among the techniques susceptible to allow the deposition of efficient anti bacterial coatings at industrial scale, reactive co sputtering is an attractive alternative method which allows the deposition at very low temperature, which foreshadows the treatment of polymers or textiles. Moreover, sputtering is a clean process for the production of nanomaterials as compact coatings. However, reactive sputtering is a non linear process which often presents instabilities at industrial scale known as hysteresis effect that causes several problems:

- very low deposition rate of ceramic coatings in the so called compound mode
- difficulty to control the coating overall stoichiometry

These drawbacks can be overcome by using a closed loop control system known as PEM (Plasma Emission Monitoring) and that consist in measuring the emission light of sputtered species representative of the target contamination and by adjusting in real time the inlet oxygen flow rate to maintain at a selected level the emission light setpoint.

In this presentation, after a short description of the industrial deposition vessel, we present the method to produce nanocomposite TiO₂-Cu or TiO₂-Ag coatings. Finally, we present some preliminary anti bacterial measurements performed on those coatings.

N°O6a-7

SECURE PROCESS THROUGH FUNCTIONNALISATION: ELABORATION OF ORGANIC COMPOSITES REINFORCED WITH ALIGNED CARBON NANOTUBES GROWN ON CARBON FIBERS

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Based on their outstanding mechanical and electrical properties, carbon nanotubes (CNTs) have opened a window to new technological approaches especially in the aerospace field to overcome the limitations of conventional composite materials and achieve higher performances. Moreover, over the last years, the development of CNTs for future commercial applications raises the issue of their potential health impacts. Potential risks could mainly come from CNTs spreading in air during handling.

In this context, we developed a Chemical Vapour Deposition (CVD) process allowing the direct growth of aligned CNTs on carbon fibres (CF), which could strengthen polymer matrix composites in order to enhance the properties through the improvement of the fibre/matrix interface. In addition, to ensure secure processes during handling and impregnation of such materials with an organic matrix, we have developed a nanometric polymer coating - obtained through chemical functionalization - based on diazonium salts [1], [2], which could also improve the chemical compatibility of CNTs with the polymer matrix.

Our process for the growth of aligned carbon nanotubes and based on DLI-CVD (Direct Liquid Injection) will be presented. It involves two successive stages performed in one global set-up by injection-CVD, with no intermediate handling between the different stages [3]: (1) depositions of a thin ceramic sub-layer on the fibre surface and (2) growth of aligned CNTs. Besides, we have optimized the synthesis process in order to improve CNT distribution over CF, enabling us to obtain large substrates (4cm*30cm) covered by CNTs. Length and density of CNTs can be controlled through different parameters such as sub-layer deposition duration or CNT synthesis conditions. In addition, we will present the chemical functionalisation of CNT grown on CF through a diazonium-based process (graftfast™ process) and the characteristics of the layers encapsulating CNT on the CF.

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N°O6a-8

CRITERIA AND GUIDING PRINCIPLES FOR THE PRECAUTIONARY DESIGN AND FOR IMPROVED RECYCLABILITY OF ENGINEERED NANOMATERIALS

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As long as results from toxicological assessments are not sufficiently accurate to warrant special legal regulation of nanomaterials, their handling should be guided by a precautionary approach. But there is a need for a preliminary assessment and for a rational implementation of the 'precautionary principle' based on sound scientific data and knowledge indicating justifiable concern.

In this contribution, the focus is placed on the developed comprehensive approach for the precautionary design and for improved recyclability of engineered nanomaterials. This approach is derived from several qualitative and semi-qualitative approaches to risk assessment and to criticality of materials, and is supplemented with environmental impact categories of Life Cycle Assessment.

Categories and aspects	Data quality	Source
Precautionary risk aspects		
Decision tree of risk categorisation	Qualitative	German SRU to precautionary strategies for managing nanomaterials (SRU 2011)
Potential exposure of humans	Semi-quantitative	Swiss precautionary matrix for synthetic nanomaterials (BAG/BAFU 2011)
Potential input into the environment	Semi-quantitative	Swiss precautionary matrix for synthetic nanomaterials (BAG/BAFU 2011)
Potential of accident	Semi-quantitative	German ÖI Sustainability check (ÖI 2011), orientation on Swiss precautionary matrix
Ressource aspects		
Criticality	Qualitative, Semi-quantitative	EU concept of criticality (EC 2010)
Recycling capability / tendency to dissipation	Qualitative	In orientation on German ÖI Sustainability check
Abiotic ressource requirement	Quantitative	LCA methodology (DIN EN ISO 14040 2006)
Other LCA impact categories		
Energy requirement	Quantitative	LCA methodology (DIN EN ISO 14040 2006)
Global warming potential	Quantitative	LCA methodology (DIN EN ISO 14040 2006)
Tox. potenzial, but not nanospecific	Quantitative	LCA methodology (DIN EN ISO 14040 2006)
Ecotox. potenzial, but not nanospecific	Quantitative	LCA methodology (DIN EN ISO 14040 2006)

Table 1: Approach for the precautionary design and for improved recyclability of engineered nanomaterials

Our approach as well as first assessment results to four nanomaterials (nanocellulose, MWCNT, nano TiO₂, and nano ZnO) and associated products in the project NanoSustain will be presented.

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N° O6a-9

FROM SAFE NANOMANUFACTURING TO NANOSAFE-BY-DESIGN PROCESSES

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Industrial needs in terms of multifunctional components are increasing. Many sectors are concerned, from the integrated direct nanoparticles production to the emerging combinations which include the matrix metal composites (MMC), ductile ceramics and ceramic matrix composites, polymer matrix composites (PMC) for bulk application in the field of automotive, aerospace for energy for surface engineering in the field of the building applications, respectively.

Moreover, domains with a planetary impact such as environmental issues, as well as aspects for instance health (toxicity)⁵ and hazard assessment (ignition and explosion severity)⁶ were also taken into account.

Nanotechnologies play an important role in promoting innovation in design and realization of multifunctional products for the future, either by improving usual products or creating new functions and/or new products. Nevertheless, this huge evolution in terms of materials could only be promoted by increasing the “*social acceptance*” and by acting on the different main technological and economic challenges and developing “*safe oriented processes*”.

Nowadays, a huge number of developments of nanoparticles are potentially industrial up-scalable. However, some doubts exist about the handling’s safety of the current technologies. For these reason, the main goal was to develop a “*self-monitored*” automation in the production line coupling different techniques in order to simplify processes such as “*in situ*” growth nanoparticles into a nanostructured matrix, over different substrates⁷ and/or the nanopowders synthesis, functionalization, dry or wet “*safe*” recovery system, granulation⁸, consolidation in “*single-step*”, by monitoring at real time the processing parameters such as powder stoichiometry⁹.

With the aim of assuring the traceability of the product during the whole life, starting from the conception and including the R&D, the distribution, the use and its recyclability were also considered.

The optimization in terms of processing, recovery and conditioning, permits to increase its versatility, thus leading to increase the add value to the production.

This review put in evidence by different examples, the progress achieved since the beginning of Nanosafe integrated initiative developed by CEA and its partners as well as promoting, firstly the safety at work place, and then extending to an integrated and controlled “*in situ*” production by of Nanosafe-by-Design controlled processes.

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N° O6b-1

**EVALUATING PERFORMANCE OF CONTAINMENT EQUIPMENT DESIGNED FOR HANDLING
MANUFACTURED NANOMATERIALS BY USE OF NANOPARTICLE MARKER**

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The implementation in many products of manufactured nanoparticles is in strong growth and raised new questions. For this purpose, the CEA - NanoSafety Platform is developing various research topics for health and safety, environment and nanoparticles exposure in professional activities.

The working group Nano-CERT/MTD, driven by INERIS, federate actors of the sector, experts, research organizations, industrial users and manufacturers of collective protection. The main activity of this group is to establish specific rules for a voluntary certification of collective protection, at a national scale, but with the possibility of a further extension at an European level. The group aims to establish an experimental protocol of certification through identification of best available techniques to characterize collective protections for workers faced with nanomaterials exposure.

The NanoSafety Platform provides a method of collective protections characterization based on the developments in nanoparticles metrology and on the study of existing standards and practices in related areas (chemicals, dust, microbiological and nuclear).

This study presents the results obtained during the experimental characterization of a potential nanoparticles transfer in a laboratory extractor hood by the use of a particulate marker of sodium-fluorescein. The efficiency of equipment and more specifically the efficiency of dynamical air barrier is evaluated, with the experimental results, by calculating the transfer coefficient (figure 1).

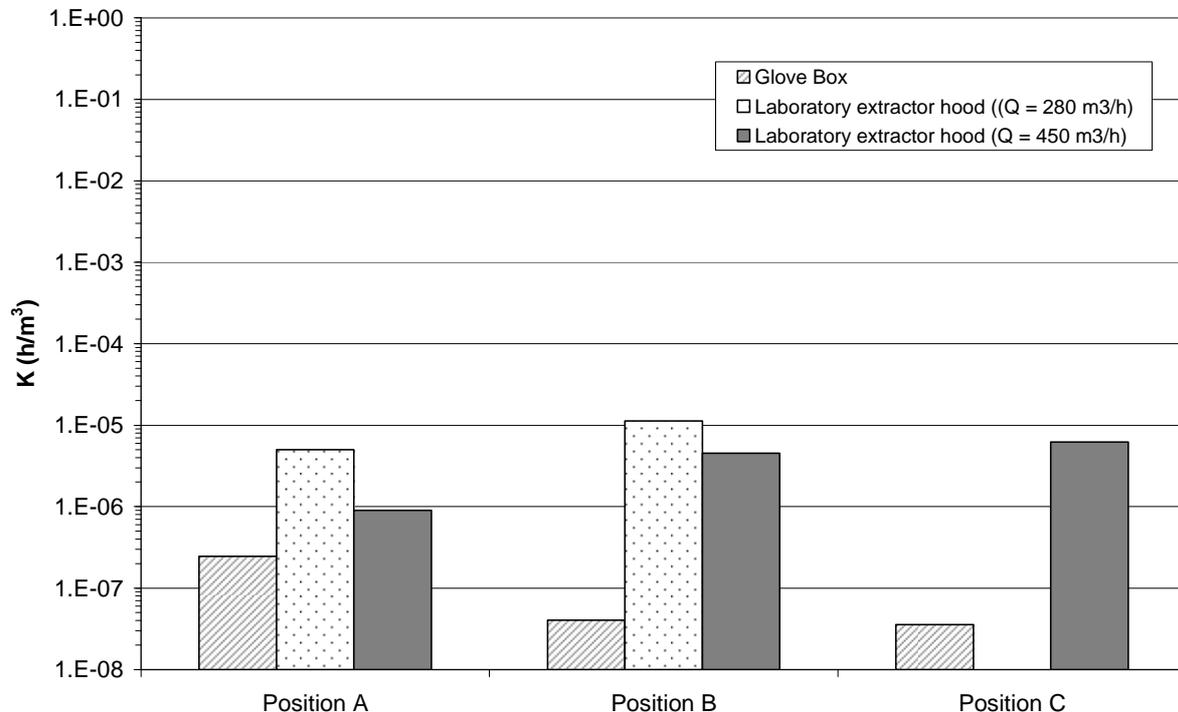


Figure 1: values of transfer coefficient for several localization of the sampler

N° O6b-2

EXPERIMENTAL AND NUMERICAL COMPARATIVE STUDY OF THE CONTAINMENT OF AIRBORNE NANOPARTICLES AND GAS RELEASED INSIDE A SAFETY CABINET

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Considering the constant increase in the use of nanomaterials, it is important to develop and employ appropriate protective devices in the event of hazardous nanoparticles release. With respect to laboratory activities, it is generally accepted that the ventilated containment enclosures (fume cupboards, safety cabinets) are appropriate for nanoparticles handling. However, there are few studies assessing independently their effective efficiencies. IRSN and INRS, through this study, seek to characterize the efficiency of such cabinets used to manipulate nanoparticles and to highlight a possible specificity in the nanoparticle behavior. The studied device is a microbiological safety cabinet (MSC), and three experimental approaches were explored as potential containment tests: a first one based on direct counting of nanoparticles (for two different types of nanoparticles, i.e. copper and sodium chloride respectively) in a clean room by means of a Scanning Mobility Particle Sizer (SMPS), a second one based on fluorescent ultrafine particles (sodium fluorescein aerosol), and a third one based on a tracer gas (sulphur hexafluoride). The backward diffusions obtained in identical configurations of emission for the three different techniques and for all considered situations are compared. The results indicate that the containment efficiency (expressed using number concentrations) obtained for an aerosol of nanoparticles is always slightly higher than the one measured for a tracer gas (the transferred quantities varying between 30% and 60% of the gas values, see Figure 1), which is consistent with the known physics of nanoaerosols. Thus, containment tests involving the generation of a tracer gas seem fit to evaluate the performance of enclosures with respect to airborne nanoparticles. Identically, fluorescein particles with ultrafine particle size distribution could be used for nanoparticle containment testing. Finally, experimental results will be supplemented with data from numerical simulations (Eulerian model).

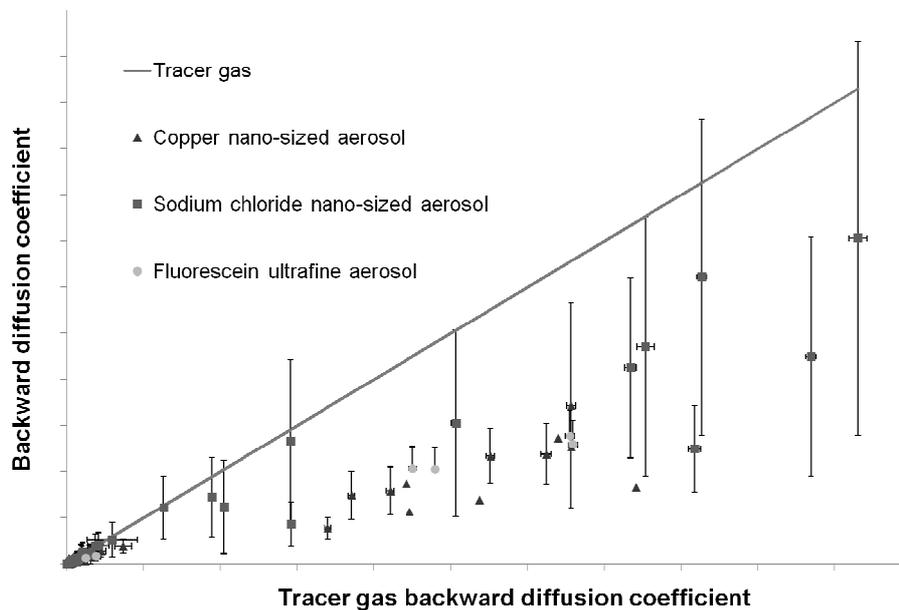


Figure 1: correlation between the backward diffusion of a tracer gas and of various aerosols in the nano and ultrafine range, for multiple evaluated situations and measurement positions.

N° O6b-3

**EXPERIMENTAL EVALUATION OF THE RESISTANCE OF PROTECTIVE GLOVES AGAINST
TITANIUM DIOXIDE NANOPARTICLES IN SOLUTION UNDER CONDITIONS SIMULATING
OCCUPATIONAL USE**

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Titanium dioxide nanoparticles ($n\text{TiO}_2$) enter in the composition of numerous manufactured products such as sunscreen or paints for example. If $n\text{TiO}_2$ use seems inescapable, some studies caution about their likely harmful effects on health. Application of the precautionary principle put forward by many Health & Safety government agencies has led to the recommendation of the use of protective gloves, however without evidence of their effectiveness. Some studies on protective gloves resistance to nanoparticles (NP) have been carried out. Yet, these tests may not correspond to the real conditions to which gloves are exposed in occupational settings.

Therefore, this study is aimed at quantifying the efficiency of protective gloves against $n\text{TiO}_2$ and evaluating the effect of real use conditions on NP penetration. Two materials were studied: nitrile and butyl rubber. They were brought into contact with $n\text{TiO}_2$ in solution and dynamic biaxial deformations were applied to the samples.

According to preliminary data, a penetration of $n\text{TiO}_2$ solutions through nitrile gloves may occur as well after prolonged dynamic deformations. No differences were observed for measurements made with or without $n\text{TiO}_2$ solutions for the samples composed of butyl rubber. Additional mechanical results show that repetitive deformations affect the physical and mechanical properties of protective gloves.

Moreover, gloves materials can be sensitive to the action of solvents used in commercial $n\text{TiO}_2$ solutions. These effects may involve the swelling of the polymer, leading to a modification of its mechanical and chemical properties. In addition, if the contact time is longer than the solvent diffusion time, it may emerge on the opposite side of the membrane, carrying NP with it. Gravimetric tests were also performed immersing gloves materials in $n\text{TiO}_2$ solutions. The swelling behaviour was assessed by mass gain and length change measurements. Results showed that the studied solutions have a significant effect on nitrile rubber. A large mass gain was recorded for short immersion times, indicating a possible penetration of the $n\text{TiO}_2$ liquid carrier in this elastomer. No effect was measured with butyl rubber.

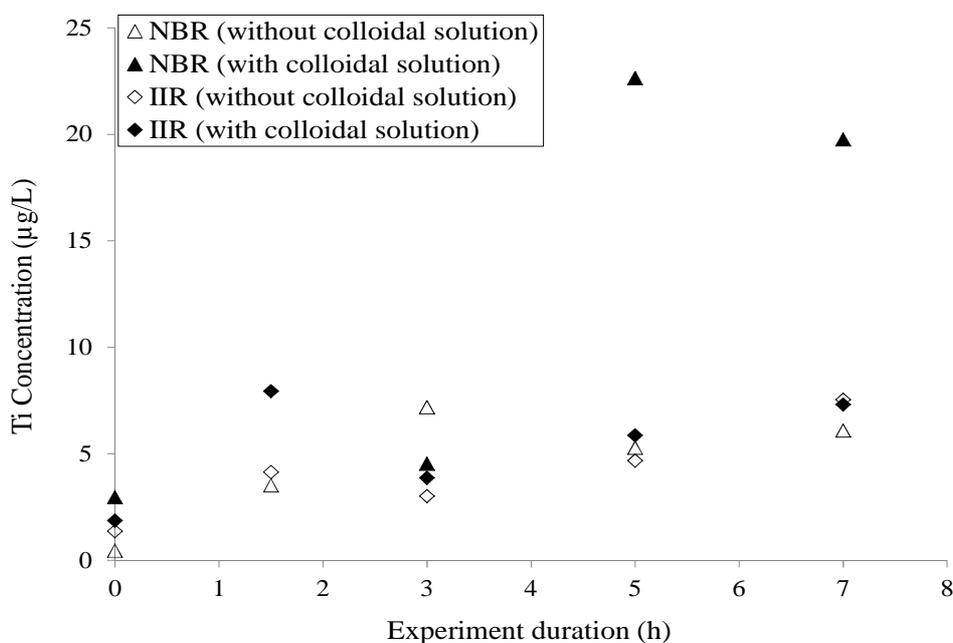


Figure 2. Concentration of titanium in the sampling solutions as a function of the duration of 50% biaxial deformation for nitrile (NBR) and butyl (IIR) rubber samples exposed to $n\text{TiO}_2$ solution in water

N°PL8

**LIFE CYCLE CONSIDERATIONS FOR ASSESSING ENVIRONMENTAL RISKS OF
NANOMATERIALS**

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The current and future widespread usage of engineered nanomaterials (ENM) in industrial applications and consumer products will inevitably cause emissions of ENM to the environment and result in an increase of human and environmental exposure. As a starting point for an exposure assessment, exploring sources and pathways of release helps to identify relevant applications and situations where humans or the environment may face exposure to ENM. By tracking the life cycle of products, it is possible to explore whether and in which situations a release of ENM from applications may occur. This presentation shows that using material flow and environmental fate modeling as basis, we can quantitatively identify the determining steps in the life cycle of nano-products that result in release. Within the environmental exposure assessment two very critical points with limited data are the knowledge about production amounts of ENM and the distribution of the ENM to different product categories. Also the release of ENM from actual products has been studied only to a limited extent. For various ENM results will be shown for release from paints and textiles and the critical points that affect the form and identity of the released materials will be discussed. Finally it is shown how exposure modeling can be linked to modeling of the environmental effects of ENM to derive a first environmental risk assessment for ENM.

N°O8a-1

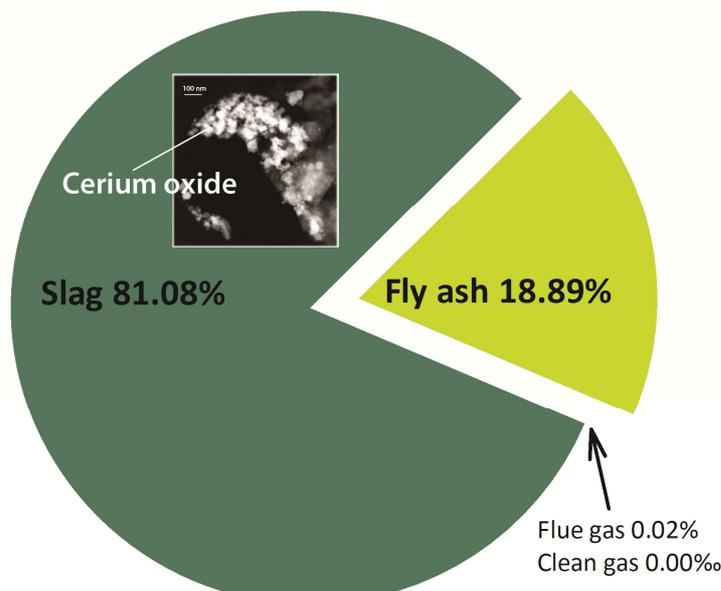
INCINERATION OF NANOWASTE: THE IMPLICATIONS FROM A LIFE CYCLE PERSPECTIVE

Tracing engineered nanooxides in a full scale waste incineration plant

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Nanoparticles and nanostructured materials are increasingly sold on the market. Their nanospecific properties allow for a wide range of new, environmentally beneficial functionalities in comparison to their conventional substitutes. Examples include increased stiffness, which allows less resource use, or higher electric conductivity, which is used in energy saving devices. As long as the nanoparticles are integrated in matrices and perform their intended function, there is little worry about potential human exposure and impacts to the environment. However, there are life cycle stages where nanoparticles are released without control and thus might enter the environment. The trend to design many different and stable nanoparticles calls for a closer look at their persistence, especially at the end of life stage of nanoenabled products. One hot spot of potential nanoparticle release to the environment is the waste incineration process, to which a large share of the nanowaste is transferred,



Cerium was mainly detected in the solid incineration residues (given in % of total detected cerium). The nano-CeO₂ was found in the form of loose agglomerates on the slag (inlet).

at least in Europe. For this reason, we investigated the so far unknown behavior of engineered nanoparticles in a full scale incinerator, using nano cerium dioxide which represents a stable class of commercially relevant nanooxides. After adding 10 kg of the 80 nm nanooxides to the waste, we collected samples of all incineration residues over 8 hours. We were not only able to test the efficiency of the flue gas cleaning system but we also quantified the nano cerium dioxide in all combustion products with state of the art analytical techniques. The nanoparticles did not reach the detection limit (0.2 ng per measurement filter) in the clean gas under worst case conditions. More than 99% of the nanoparticles were transferred to the slag and fly ash which are further processed for

material recovery and landfill. Surprisingly, the nanooxides were found unchanged in loose agglomerates in the slag. While modern incineration plants can keep the nanoparticles from being released to the atmosphere, future studies should take a closer look into the processing of solid waste incineration residues. The results of the conducted human exposure studies inside the waste incineration plant point towards an exposure to engineered nanoparticles at certain working procedures (maintenance of installations, periodic sampling of residues). In addition to the self-evident worker protection measures, there is a requirement for further exposure studies in waste incineration plants as well as in the processing of solid incineration residues.

N°O8a-2

**PRELIMINARY EVALUATION OF RISKS RELATED TO WASTE INCINERATION OF POLYMER
NANOCOMPOSITES**

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If nanotechnology proves to be successful for bulk applications, large quantities of nanocomposites are likely to end up in municipal solid waste incineration (MSWI) plants. Various studies indicate that nanoobjects might be harmful to human health and the environment. Possible health effect concern inhibition of the immune system, respiratory toxicity, cardiovascular toxicity, toxicity to the nervous system and brain and damage to cells and DNA. Also it has been shown that nanoobjects exhibit ecotoxicity. Because of the possible health risks of nanoobjects, exposure to humans should be prevented and nanoobjects should not be released into the environment. At this moment there is no evidence that all nanoobjects are safely removed from the off-gas when incinerating nanocomposites in MSWI plants. A preliminary assessment of the fate of nanoobjects during waste incineration and the ability of MSWI plants to remove them is presented. It appears that nanoobject emission levels will increase if bulk quantities of nanocomposites end up in municipal solid waste. Many primary and secondary nanoobjects arise from the incineration of nanocomposites and removal seems insufficient for objects that are smaller than 100 nm. For the nanoobjects studied, risks occur for aluminum oxide, calcium carbonate, magnesium hydroxide, POSS, silica, titanium oxide, zinc oxide, zirconia, mica, montmorillonite, talc, cobalt, gold, silver, carbon black and fullerenes. Since this conclusion is based on a desktop study without accompanying experiments, further research is required to reveal which nanoobjects will actually be emitted to the environment and to determine their toxicity to human health.

N°O8a-3

SAFE AND ENVIRONMENTALLY-FRIENDLY MANAGEMENT AND FINAL ELIMINATION OF WASTES HARBORING NANO-OBJECTS, OR LIKELY TO RELEASE NANOPARTICULES

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So far, there is no specific approved procedure or regulation for the management and final elimination of wastes either containing nano-objects or likely to release nano-particles, mainly due to the unrecognition of a "nano-specificity" of such materials. As recommended by the French sanitary security agency (ANSES), nanoparticles should be considered as presenting an "*unknown danger level*", and, as a consequence, they should be handled by applying the same safety procedures as those used to manage **hazardous wastes**, *i.e.* residues that may pose a substantial hazard (actual or potential) to human health or the environment when improperly stored, transported, treated or disposed of.

Two safe industrial pathways are already available within Séché Environment to treat such specific "nano"wastes, based on their composition :

- i) specialized incineration within our Hazardous Wastes Incineration Plants, when the wastes mostly contain combustible organic compounds;
- ii) landfilling of inert inorganic residues in our Hazardous Wastes Storage Facilities (ISDD), under conditions similar to those recommended for free asbestos, *i.e.* with a preconditioning in double-walled UN approved big-bags, and with a possible pre-solidification step with hydraulic binders.

Herein, we describe the best practices and organizational procedures implemented within Séché Environnement facilities for the safe and environmentally-friendly management and final elimination of such potentially hazardous "nano"wastes, either containing nano-objects or likely to release nano-particles. Through our industrial pathways, potentially hazardous handling operations like deconditioning, grinding or mixing are whenever possible limited, and when strictly necessary, they are performed under controlled sealed atmosphere, on specific containment areas within our duly authorized hazardous wastes treatment plants, equipped with extensive dusts-collection equipments and water injection systems. Swept-air and dust-laden waters are collected and conveyed to specific pollution control devices. Any human manipulation is performed by wearing fully-sealed overall body "hazmat suits", equipped with breathing air supply.

A recent study by Walser *et al.* (Nature Nanotechnology, 2012, doi:10.1038/nnano.2012.64 279) reported no emission of Cerium (sub-detection levels) in the exhaust fluegas when introducing significant amounts of engineered nano-CeO₂ in a Swiss municipal wastes incineration plant. This result provides confidence about the efficiency of the current sophisticated air pollution control devices. But the authors also pointed out the persistence of engineered nanoparticles in the solid residues recovered from the incineration process (bottom and fly ashes), thus highlighting *i)* the necessity to consider such inert residues as hazardous wastes for their safe final disposal, and *ii)* the limit to incinerate "nano"wastes in non-specialized thermal treatment plants, like a municipal thermal treatment plant, among non-hazardous household wastes.

Similarly, the French NanoFlueGas project (2011-2014), supported by ADEME and gathering INERIS, TREDI-Séché Environnement and the Ecole des Mines de Nantes-GEPEA, is devoted to describe the fate of nanoparticles during the incineration of real "nano"wastes at both small- and real-scales.

N°O8a-4

**ENVIRONMENTAL EXPOSURE MODELLING OF ENGINEERED NANOMATERIALS AND
COMPARISON TO THEIR CORRESPONDING BULK/TOTAL MATERIAL FLOWS**

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There is a clear necessity to assess the environmental risks of engineered nanomaterials (ENM) due to their rapidly increasing application in consumer products, which might lead to an increasing environmental exposure. Using probabilistic material flow modelling, we have reported in 2009 the first environmental concentrations for different ENM in various environmental compartments¹. The present study aims to present more comprehensive and up to date environmental concentrations of ENM by taking advantage of the much more comprehensive data on production, use and behavior of ENM available in 2012. The evaluation also includes a comparison of the ENM concentrations to their corresponding total conventional materials, e.g. pigment-TiO₂, Silver, Zinc and Carbon black. The analysis was conducted in two stages: (1) modeling updated material flows and environmental concentrations of five ENM (nano-TiO₂, nano-ZnO, nano-Ag, Carbon Nanotubes (CNT) and Fullerenes (C₆₀)); (2) modelling the material flow and environmental concentration of pigment TiO₂ and summarizing the total concentrations/flows of conventional Ag, Zn and Carbon black. Our modeling was based on newest available data for production volumes of ENM, allocation of ENM to product categories, release of ENM from products and flow coefficients into / within environmental compartments. For environmental concentrations of pigment TiO₂ a similar probabilistic material flow modelling was performed. The comparison to total Ag, Zn and Carbon black was based on information from the literature.

The modeled production volumes in 2012 are mostly higher than those of 2009, which matches well to the development of nanotechnology, e.g. the modeled most-probable nano-TiO₂ production in the EU in 2009 was 3393 tons, and the value for 2012 is 9930 tons. However, for nano-Ag the production in 2012 was modeled to be lower than the assumption from 2009, reflecting a better knowledge on production amounts. The predicted environmental concentrations for 2012 are in most cases 2-4 times higher than the results of 2009. This is caused to some extent by the higher production amount but also by better knowledge on some transfer factors that resulted in more conservative estimates on their behavior.

The modeled most-probable pigment-TiO₂ production in the EU in 2012 is 1'443'000 tons, which is about 3 magnitudes higher than that of nano-TiO₂. The ENM flows are between 1 (for nano-Ag) to 4 (for CNT) orders of magnitude smaller than the flows or concentrations of the corresponding conventional materials. These comparisons will enable a more realistic assessment of the risks of ENM to the environment by focusing not only on the "nano" issue but taking a more complete view and relating the ENM to already existing compounds.

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N°O8a-5

**LCA MODELLING OF ENGINEERED NANOMATERIALS:
A FRAMEWORK FOR ESTABLISHING SOUND INVENTORY DATA OF PRODUCTION &
RELEASES OF NANO OBJECTS ALONG THE LIFE CYCLE**

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From several research groups it was shown in recent publications that there are actually only few Life Cycle Assessment (LCA) studies of engineered nanomaterials (eNM) published so far; studies that are moreover far away from what is considered nowadays “comprehensive” and “sound” LCA studies. Thus, I am currently developing a generally applicable framework for a comprehensive and adequate modelling of the production of eNM, taking into account possible emissions of nanoparticles to the environment (not only in the production, but along the complete life cycle), and translate this framework then into exemplary life cycle inventory (LCI) datasets for the production of eNM.

Actually, within this framework two topics of concern and their different strategies have to be distinguished – (a) the modelling of conjunctions with technosphere (i.e. the direct material and energy inputs into the production process of eNM), and (b) the modelling of releases of nano objects during the various life stages.

For the first element, a thorough analysis of the different approaches for the modelling of chemical production processes within the ecoinvent database concerning their applicability in the area of the production of eNM has been established. This analysis shows clearly that due to their broad process variability (including the yield of the used technologies), only a case-specific unit process data collection strategy can work; resulting – when following the quality requirements from the ecoinvent Centre – in transparent, comprehensive and adequate gate-to-gate inventory datasets; datasets that can then easily be integrated into a variety of different database systems with background LCI data. However, due to the fact that most production processes of eNM are today (still) proprietary processes - the fulfilment of the term ‘comprehensive’ asks still considerable efforts. An exemplary case study for this point will be shown during the presentation.

Concerning the second issue, I currently develop in my (on-going) research a stepwise procedure for the identification of those properties that are required in order to model emissions of nano objects in an adequate and compatible manner with the current practice in LCI/LCIA modelling. The objective of my work there is an identification of existing relationships between the properties of emissions of nano objects and the relevant parameters defining environmental effects that are concerned by this type of emission (i.e. toxicological aspects). In the presentation the outcome of this, at the moment on-going work, and its consequences for a simple case study from the ICT sector will be shown.

N° O8a-6

**LCA CASE STUDIES OF NANOTECHNOLOGY-BASED APPLICATIONS IN THE PROJECT
NANOSUSTAIN**

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Nanotechnology is frequently described as an enabling technology and fundamental innovation, i.e. it is expected to lead to numerous innovative developments in the most diverse fields of technology and areas of application in society and the marketplace [1, 2].

In this contribution, the focus is placed on the current status of the LCA case studies in the project NanoSustain. The project NanoSustain investigates the following specific organic and inorganic nanomaterials and associated products:

- nanocellulose based materials and products (paper additive, industrial thickener, rheology modifier),
- MWCNT based products (epoxy plates),
- nano TiO₂ based products (paint application), and
- nano ZnO based composites (glass coatings).

Life Cycle Assessment (LCA) is the most extensively developed and standardised methodology for assessing the environmental aspects and potential impacts throughout a product's life from raw material acquisition through production, use and recycling and/or disposal (i.e., cradle-to-grave). Life Cycle Assessment, like all methodologies, also has its weaknesses, blind spots, and deficiencies especially for the assessment of nanotechnologies. Current review publications to the state-of-the-art of LCA of engineered nanomaterials clarify also impressively the existing methodical gaps with the use of LCA at the nanoscale [3, 4, 5].

For modeling, calculation, visualization, and evaluation of material and energy flows together with environmental impacts, the flexible and powerful LCA software tool Umberto are be used. The life cycle assessment based on the one hand of data from the project partners and literature, and on the other hand of datasets of the LCA databaseecoinvent from the Swiss Centre for Life Cycle Inventories. Additionally, different assumptions have been made for technological developments, some process steps, chemical synthesis and, after use phases [6, 7].

Our approach of the modeling as well as first project results of the case studies will be presented. In addition problems will be discussed during the data acquisition etc for nano-specific emissions, especially in use phase and the after use phase (recycling and/or final treatment and disposal) of the products.

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N° O8a-7

**LIFE CYCLE ASSESSMENT OF A SELF-CLEANING COATING BASED ON NANO TiO₂-
POLYUREA RESIN APPLIED ON ALUMINUM PANEL**

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Traditionally, titanium dioxide materials have been used as a white pigment. With development of TiO₂ nanomaterials, applications have greatly expanded to other areas, such as cosmetics, energy, and photocatalysts. TiO₂ nanomaterials have applications in diverse areas, including ultraviolet (UV) protection, photocatalysts, photovoltaics, sensing, water treatment, antibacterial agents in medical devices and air purification. Titanium dioxide is one of the most important and common photocatalyst because of its outstanding efficiency (even under weak solar irradiation), compatibility with a large number of materials and good stability. Moreover the photocatalytic reaction of TiO₂ is very effective to decompose organic matter. The UV light induces superhydrophilicity of TiO₂, so it can be applied to solid surfaces to obtain self-cleaning effect. TiO₂ nanoparticles are being applied directly or added to liquid media (paints, resins, glazed, etc.) and successively sprayed on surfaces to exterior and interior coverings and/or walls of buildings to provide a self-cleaning, de-polluting and self-sterilizing functions. The present LCA study concerns the application of polyurea resin functionalized with TiO₂ nanoparticles on an aluminum panel with spraying system in order to define the most critical aspects of the process and to minimize the environmental burdens. The system function is the application in cleanrooms typically used in manufacturing or scientific research, operating room and in all environments that require low level of pollutants such as dust, airborne microbes, aerosol particles and chemical vapors. The functional unit chosen for this study is 6 kg of polyurea resin functionalized with TiO₂ nanoparticles, that is the amount of coating applied on aluminum panel. The LCA study has been performed using the SimaPro 7.3.3 software and IMPACT 2002+ impact assessment method. The system boundaries for the analysis include the upstream phases, from raw material extraction to panel recycling, thus obtaining “a cradle to the grave” overview. In this study a preliminary attempt to define the damage generated by the emissions of titania nanoparticles during the use and end of life phases have been reported. LCA analysis indicates that the total damage is 0.0378 Pt. The highest environmental impacts are mainly due to aluminum panel (24,78% of the total damage) and consequently to isocyanate and amine resin used in the process to produce the polyurea resin (12,95% and 11,35% respectively of the total damage).

Damage category	Unit	Total	Amine resin	Isocyanate	Aluminium panel
Total	Pt	0,0378	11,35%	12,95%	24,78%
Human health	Pt	0,0115	9,26%	14,24%	20,08%
Ecosystem quality	Pt	0,0035	3,40%	1,82%	54,68%
Climate change	Pt	0,0090	10,57%	12,76%	24,17%
Resources	Pt	0,0111	15,41%	17,30%	21,84%
Radioactive waste	Pt	0,0027	16,24%	4,56%	20,88%

Evaluation by damage categories of 6kg of polyuria resin functionalized with nanoparticles

N°O8a-8

IMPACT ASSESSMENT OF NANO ACTIVATED POLYMERIC MEMBRANES

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The use of nanoparticles in the manufacturing process of polymeric membranes has received much attention during the last years due to a high degree of control over membrane fouling and the ability to produce desired structures and new functionalities. The latest membrane morphology emerging with the potential for future applications involves layer by layer (LbL) process and mixed matrix membrane (MMM). LbL involves an alternating deposition of oppositely charged polyelectrolytes layers on a charge membrane surface to enable potential attachment of nanoparticles on it mostly by electrostatic forces. MMM consists typically of a bulk polymer phase wherein the dispersed phase is the inorganic nanoparticles. LbL and MMM have the potential to achieve higher selectivity, permeability, or both relative to the existing polymeric membranes, resulting from the addition of the inorganic nanoparticles with their specific properties. However, the increasing use of nanoparticles in different consumer products has raised certain concerns over their safety to human health and the environment. Every nano-based solution must not only be cost-effective, but also be sustainable and safe for people and the environment.

Within the EU-funded NAMETECH project (*Development of intensified water treatment concepts by integrating NAno- and MEmbrane TECHnologies*), a Life Cycle Thinking was applied to investigate potential impacts and benefits posed by the use of nanoparticles in membrane filtration systems. Particularly, a cradle-to-gate life cycle assessment (LCA) has been performed to: (i) evaluate the potential impacts due to the integration of titanium dioxide nanoparticles (nanoTiO₂) in polyethersulfone (PES) membrane by LbL technique, and (ii) compare nanoTiO₂ MMM with conventional membrane. The environmental performance of the following process has been investigated: PES membrane manufacturing (spinneret process), electrostatic deposition of nanoTiO₂ on PES membrane activated by LbL, synthesis of PES / nanoTiO₂ MMM in comparison to traditional PES/polyvinylpyrrolidone (PVP) membrane. Inventory data were collected from the NAMETECH partners and integrated with literature, and the database included in the LCA software used (SimaPro 7.1). The results show that the deposition stage of nanoTiO₂ on membrane has a very negligible effect on all selected impact categories, in comparison to the various stages investigated during the manufacturing of PES membrane (fig. 1). The electricity consumption during the membrane production, as well as the solvents and polymers needed for making PES membranes are the main contributors to the overall environmental impact.

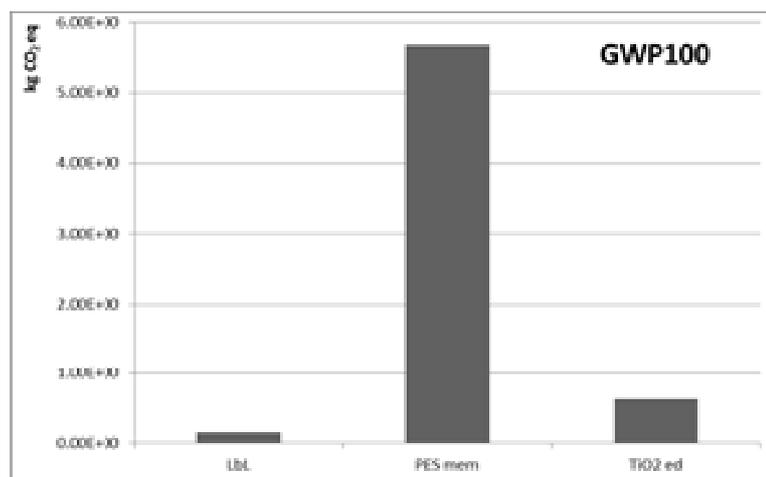


Figure 1. Results of the characterization of environmental impacts for PES membrane activated with nanoTiO₂. The contribution of global warming potential for the time horizon of 100 years (GWP100) is shown. Legend: LbL=Layer by layer; PES mem=PES membrane manufacturing; TiO₂ ed: electrostatic deposition of nanoTiO₂.

N°O8a-9

INVESTIGATION OF THE LIFE CYCLE OF NANOPARTICLES BY MEANS OF [^{44,45}Ti]TiO₂ AND [^{110m}Ag]Ag⁰ – RESEARCH PROJECT NANOTRACK

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Production and application of nanocomposites such as functional surface coatings have significantly increased in recent years. Nanoparticle (NPs) coatings are used in a wide array of applications ranging from self-cleaning and scratch resistant surfaces to biocidal coatings. The current database for risk evaluation of NPs containing surface coatings (e.g. TiO₂, Ag⁰) is still insufficient. Tools are currently lacking with which to assess the impact of TiO₂ and Ag⁰ NPs. Radiolabeling of the NPs provides a method to sensitively detect NPs and is feasible for qualitative and quantitative fate and effects determination. With this detection method, evaluation of NPs fate during aging and abrasion of nanocomposites, estimation of release rates, transport of NPs in the environment and up-take and effects with organisms can be studied in great detail.

The joint research project NanoTrack uses model surface coatings in an acrylate-based formulation containing TiO₂ (d = 21 nm, P25, Evonik Industries) and Ag⁰ NPs (d < 100 nm, Sigma Aldrich). Coatings were produced by application of 25 µm thick nanocomposite layers (thickness of wet coat) on a substrate followed by curing and later weathered under laboratory standard test conditions. Due to the low resistivity of this model system, the organic matrix of the surface coating was severely degraded and nanoparticles were partly released. Scanning electron microscopy showed that mostly aggregates and agglomerates of NPs were released and only a small fraction of primary NPs can be expected to be discharged. Nevertheless, further environmental processes can also lead to disaggregation and stabilization of smaller NPs composites.

Current studies on the environmental fate and effects of nanoparticles are limited by our inability to detect and quantify nanoparticles in complex environmental test systems and radiolabeling nanoparticles may provide a solution to this limitation. Isotopic labeling was carried out via a low-temperature diffusive implementation of radionuclides resulting in [⁴⁴Ti]TiO₂ and [^{110m}Ag]Ag⁰ NPs (Hildebrand & Franke, 2012). Chemical composition, particle size distributions and morphology of the radiolabeled NPs remained unaltered compared to the original material. Upon suspension in various test solutions, [⁴⁴Ti] from TiO₂ and [^{110m}Ag] from Ag⁰ NPs did not leach from the NPs and remained within the lattice framework of the NPs. Within the project, interactions of the NPs with environmental geological media (such as humic acids or sediments) and transport in flow through systems are under study.

Another important aspect is the ecotoxicological impact of the released NPs. In case of entry of the NPs in aquatic systems, interactions with living organisms become very likely. Biofilms are considered as potential receptor of industrial nanoparticles in the environment and as an important part of aquatic ecosystems it is not yet known if these NPs may end up in higher organisms via the food chain transfer. Systematic studies of NPs behavior in aquatic systems are carried out to gain knowledge of their fate and transport and potential risks for ecosystems.

The integrated examination of NPs in surface coatings in terms of production, aging and abrasion, NP release and their fate and transport in the environment provides a data base for risk assessment and validation or possibly adaptation of new nanocomposite production.

Hildebrand H and Franke K (2012) J Nanopart Res 14:1142.

N°PL9

NANOTECHNOLOGY AND THE NARRATIVES OF RESPONSIBILITY

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We examine how the meaning of 'responsible innovation' might be better understood by analogy with two existing, future-oriented varieties of responsibility, namely individual parental and collective political responsibility. We examine the individual and collective 'virtues' necessary to exercise the quasi-parental and political forms of responsibility contained in responsible innovation, along with some of the ways in which cultural narratives can help us understand both the complexities involved in moral choice about technologies, and the limits of our capacity to take responsibility.

Références

A.Grinbaum and Ch. Groves, "What is responsible about responsible innovation?", in Responsible Innovation, ed. Richard Owen, Wiley Academic Publishers (in print).

N°O9a-1

NANOTECHNOLOGY, RESPONSIBILITY AND RESPONSIBLE INNOVATION

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The concept of responsible innovation (or “responsible research and innovation”, as per Von Schomberg 2011) has become much discussed in relation to emerging technologies in general and nanotechnology in particular. What is signified by the “responsible” in “responsible innovation” is by no means obvious, however. To whom and for what are researchers and innovators in nanoscience and nanotechnologies to be responsible? The difficulties with specifying answers to these questions are evident in the definition of accountability given by the European Commission in 2008 in its Code of Conduct on responsible nanotechnologies research:

“Researchers and research organisations should remain accountable for the social, environmental and human health impacts that their N&N research may impose on present and future generations” (p. 8)

Here, a precautionary impulse leads to a statement which is deeply problematic: should researchers really be accountable for *all* the impacts of their research, on both present and all future generations? In the face of such statements, it is arguably urgent to reinscribe limits around responsibility and accountability, and thus to define more precisely what they are to signify. Yet at the same time, there is a need to acknowledge a truth at the heart of the precautionary worldview – namely, that technological innovation, being a future-oriented activity that transforms the world around us in novel and sometimes unanticipated ways, creates uncertainties which limit the usefulness of traditional consequentialist or deontological ethical principles in determining what should count as responsible innovation. In this talk, I outline more precisely the reasons for the ethical difficulties inherent in talking about responsible innovation, along with the coordinates of an ethical research programme intended to be an adequate response to these difficulties.

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N°O9a-2

**THE SOCIAL CONTEXT OF NANOTECHNOLOGY AND REGULATING ITS UNCERTAINCY: A
NANOTECHNOLOGIST APPROACH**

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With 1317 consumers' products already registered on the database of the Project on Emerging Nanotechnology, the use of Nanotechnology is a reality on the market but it is only the emerging part of an iceberg. The potential exponential increase of nanotechnology uses is starting to worry major Non-Governmental Organisation (NGO's), other organisation and regulating agencies due to the uncertainty inherent of every emerging technology.¹

By definition, Nanotechnology is a technology that permits the manipulation of matter on an atomic and molecular scale (generally with materials with at least one dimension sized from 1 to 100 nanometres). According to the number of the publication on safety/toxicity of nanomaterial, it seems that most of the health concerns related to nanotechnology are about nano-objects, which may enter the environment via the air or water and be inhaled or ingested. In the other side there is much less concerns in the development of nanoelectronics probably because these electronic components do not become airborne and are directly fabricated on a semiconductor chip, with macroscopic size.² Similarly to all toxic components of electronic apparatus, the isolation of the toxic product from consumer's exposure may permit its safe use. Therefore, concern seems to be focus on nanomaterial that may release free nano-objects. Here, major concerns from a nanotechnologist point of view are:

- Corrosion of metallic nano-objects
- Aggregation to sizes that trigger immune exacerbation
- Presence of toxic by-standards
- Biological membrane/barrier perturbation
- Protein association & protein alteration

We are now facing a "paralysis by analysis"³ situation. The description of phenomena occurring at the nanoscale has not been classified yet, thus while there is contradictory studies on "unknown" samples regulators agencies have no idea where "nano" start or even if it exists, and have no strategy to address these issues yet.⁴ To answer social concerns the development of Nanotechnology need to provide full and accessible information on new product, establish the safety at the first stage of nanomaterial design and attempt to restrict the risk at the production site where safety monitoring is easily controllable.

It is therefore necessary to stop with the "seek and try" technique and start the effective and potential "bottom up design" where the properties (and principally safety features) could be determinate from the design of the material directly from the nano-scale as well as help to calibrate/understand the risk and benefit of advanced nanomaterials.

¹ <http://www.cnbs.eu/index.php/editorial/item/37-uncertain-regulation-uncertain-future>

² From interview of Wolfgang Porod, Ph.D., director of Notre Dame's Center for Nano Science and Technology by SouthBendTribune « Nanotechnology and safety: truths and misconceptions ».

³ S. F. Hansen, A. Baun, **When enough is enough**. Nature Nanotech. (2012) 7, 409–411.

⁴ EPA Report No. 12-P-0162 : « EPA Needs to Manage Nanomaterial Risks More Effectively »

N°O9a-3

THE NANO@SCHOOL PROJECT: A NEW PEDAGOGICAL INITIATIVE TO INCREASE THE AWARENESS OF NANOSCIENCES AND NANOTECHNOLOGIES IN THE CLASSROOMS

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Each year, more than 350 high school students participate in nanotechnology labs, while 80 teachers follow two days of continuing education training. The objective is to develop outreach programs on micro and nanotechnologies using an attractive educational approach based on active student participation and on the use of state of the art technologies. This initiative takes advantage of the unique economic and scientific environment of the MINATEC campus and the support of world class universities (UJF, G-INP). This program also builds on collaborations with the French high school administration (L'Education Nationale), whose goal is to set up modern physics and chemistry programs in coherence with front-line research, thereby allowing students to participate in informed debate.



N°O9a-4

LATEST RESEARCH RESULTS ON THE EFFECTS OF NANOMATERIALS ON HUMANS AND THE ENVIRONMENT: DANA - KNOWLEDGE BASE NANOMATERIALS

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Nanotechnology is considered one of the key technologies of the 21st century. The success of this fascinating technology is particularly based on its versatility. It will bring about fundamental changes of basic research as well as of many sectors of industry and also of daily life from electronics to the health care system. However, many consumers miss reliable and understandable information on nanomaterials and nanotechnology, e.g. on the basic questions:

What exactly are nanoparticles? What is meant by "exposure"? When do toxicologists speak of a risk?

These and many more questions are answered by our knowledge base: www.nanoobjects.info.

In an interdisciplinary approach, scientists from different research areas, such as human and environmental toxicology, biology, physics, chemistry, and sociology provide a knowledge base for more transparency. The DaNa project team processes the results of research on nanomaterials regarding their influence on humans and the environment in an understandable way.

For this purpose, we integrate results of projects (funded by the German Federal Ministry of Education and Research), analyse scientific publications, reports, and latest news on human and environmental toxicology. The state of knowledge is wrapped up in the knowledge base. Literature providing the basis of the nanomaterials knowledge base is selected in accordance with quality criteria that have been acknowledged worldwide within the scientific community. For this we developed a "Literature Criteria Checklist" to evaluate publications. Standard Operation Procedures (SOPs) should be a basis for future projects and publications. On the basis of careful scientific practice, the DaNa project team has compiled a SOP template to fill in. Both the Literature checklist and the SOP template are available on our webpage for download.

Additionally, DaNa provides a list of FAQ, a glossary defining nano-relevant terms, a dialogue platform with contact data to other information platforms and the opportunity to ask questions to our experts via mail. DaNa is also present on Twitter, follow us: @nano_info.

DaNa is a German umbrella project funded by the German Federal Ministry of Education and Research and supported by Swiss Federal Authorities.



N°O10a-1

OPTICAL INSTRUMENTATIONS FOR NANOSAFETY

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The French company HORIBA Jobin Yvon SAS, founded in 1819, specialized in optical spectroscopy, is the European center of the HORIBA Scientific segment.

HORIBA Scientific designs and manufactures scientific instruments in the fields of photonics and biophotonics. The range of instruments developed (Raman spectrometers, fluorescence spectrometers, particle analyzers, ellipsometry, GDS-OES, ICP-AES, sulfur and chloride analyzers in hydrocarbons, analysis of water quality, XRF, ...) allows a detailed characterization of samples in the academic and industrial sectors. In 2009, the acquisition of the company GenOptics completes the technological portfolio of HORIBA Scientific by integrating Surface Plasmon Resonance imaging (SPRi) for the analysis of biomolecular interactions without labeling.

The application fields of these technologies are semiconductors, biotechnology, environment and medicine in the basic and applied research.



www.horiba.com/scientific

N°O10a-2

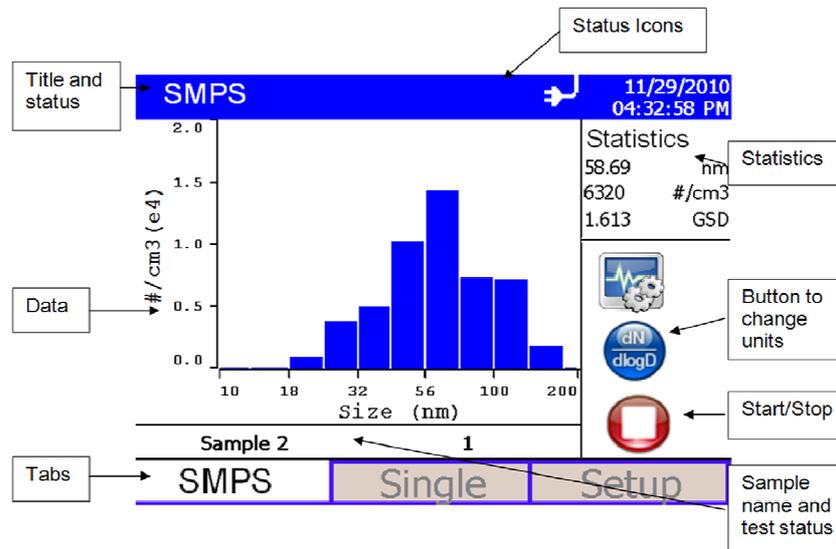
NANOSCAN SMPS - A NOVEL, PORTABLE NANOPARTICLE SIZING AND COUNTING INSTRUMENT

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Sources of nanoparticles are manifold and include a large variety of anthropogenic sources like engineered nanoparticles and particles from combustion and industrial process emissions. However, nanoparticles are also present in ambient air as well as in indoor environments. A key parameter for the characterization of particles is their size distribution and number. Recent health studies indicate an important role of particle number and surface. Measuring exposure to nanoparticles was mostly up to academic research using high end technology, this may not be necessary to that degree for a lot of applications, e.g. in the field of Indoor Air Quality and Occupational Health and Safety.

We are introducing a novel, portable nanoparticle sizing instrument, the NanoScan SMPS. It has been designed for multiple applications such as monitoring of industrial process emissions and workplaces, mobile studies, in-vehicle and outdoor air quality measurements. Its measurement technique is based on the electrical mobility sizing of a Scanning Mobility Particle Sizer (SMPS), using a Radial Differential Mobility Analyzer. The size classified particles are individually counted by an integrated, isopropanol-based Condensation Particle Counter (CPC). Number size distributions in the range from 10 to 420 nm can be obtained. The size range can be extended to coarse particles with an additional compact and portable instrument, the Optical Particle Sizer (OPS), which allows covering of the full range from 10 nm to 10 µm. The NanoScan SMPS is ideal for portable applications, it includes a built in computer and is battery-powered. The device is easy to use and without any hazardous substances (no butanol, no radioactivity) that could restrict the use to designed areas.

We present measurements from different sources and locations, including several test aerosols generated in the laboratory, emissions during material testing and processing and a mobile study. The novel NanoScan SMPS compares well with established methods and size measurements such as the research-grade SMPS from TSI. Results from measurements and applications will be presented, which demonstrate the performance and capability of this easy tool to identify nanoparticle emissions and increase the workplace safety by reliable mobile measurements of number-size-distribution.



NanoScan SMPS results of a particle size distribution measurement in SMPS mode (1 min scan). All features of the NanoScan SMPS color touch screen display are shown and explained as well.

N°10a-3

DETECTING & CHARACTERIZING NANOMATERIALS IN COMPLEX MATRICES & AIRBORNE

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Critical research is being conducted to quantify the potential benefits of nanomaterials as they are integrated into composites and used as drug delivery vectors and biomarkers. Important efforts are also ongoing to better understand the effects these materials on the environment and population. This work requires an ability to detect, observe and characterize these nanomaterials in their natural form as they interact with other materials and biological matrixes, including cells, tissue and whole animal organisms. Additionally, a fast, accurate method for detecting the presence of airborne nanomaterials is also required. A specialized hyperspectral microscope technology has been specifically developed to support these research needs. This technology utilizes patented darkfield-based illumination optics, creating high signal-to-noise images of nanomaterials interacting with both biological and materials samples. The integration of hyperspectral imaging with this high signal-to-noise microscopy technology allows the creation of high resolution spectral images of these samples. This enables the characterization of individual nano-particles based on their chemical composition and added functional groups. It also enables the ability to spectrally confirm the presence and location of nanomaterials as they are integrated into multiple environments. Examples illustrating the use of this technology with multiple nanomaterials applications will be presented.

N°O10a-4

HIGH RESOLUTION PORTABLE SCANNING MOBILITY PARTICLE SIZER DESIGNED FOR BEST PRACTICE NANO EXPOSURE MEASUREMENTS

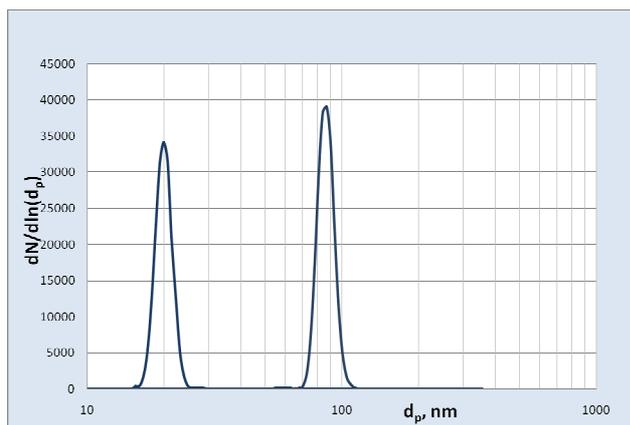
Brian Steer^{1,2}, Boris Gorbunov¹, Robert Muir¹. (1) Naneum Ltd., Canterbury Innovation Centre, University Road, Canterbury, Kent, CT27FG, UK. (2) School of Physical Sciences, University of Kent, Canterbury, Kent, CT27FG, UK

Scanning Mobility Particle Sizers (SMPS) have now been a standard tool of aerosol science for many decades. These instruments have traditionally been large, heavy, desktop devices mainly suitable for laboratory use. However in recent years there has been growing demand for smaller, portable instruments that can be freely and easily moved to sample different aerosols in situ; desirable particularly in the fields of occupational hygiene and environmental monitoring. To meet emerging requirements such as a tiered approach to monitoring workplaces a high resolution instrument is necessary. The Nanoparticle Spectrometer NPS500 from Naneum has been designed to meet these requirements in a portable, easy to use instrument.

The DMA (Differential Mobility Analyser) used in the NPS500 is based upon a novel parallel plate geometry and incorporates a unique aerosol inlet / outlet configuration that together, provide higher resolution than standard commercially available cylindrical shaped DMAs. The design was based upon finite-element modelling that produced an optimal DMA geometry; it yields the highest possible resolution in a miniaturized form factor suitable for use in portable instrumentation.

By comparison; the most common DMA design used in commercial DMA and SMPS instruments is a cylindrical column. The physical size and form factor of column styles is suitable for laboratory / bench top style instruments. These designs however, are not practical for use in a compact, self-contained and portable instrument. The DMA used in the NPS500 has been designed to provide exceptional sizing and resolution while at the same time being capable of integration into a compact, portable instrument. The sizing accuracy and resolution of the DMA in the NPS500 has been tested against standard laboratory references and with standard particles.

The resolution of the NPS500 DMA has been shown to be < 5% which is more than three times better for the same ratio of flow rates than that for a leading manufacturer. Sizing accuracy is 2% which compares favourably with similar commercial instruments.



An example of two aerosol size distributions obtained with NPS500 for Cr₂O₃ nanoparticles fractionated with the PMC500 Particle Mobility Classifier. Note – each distribution was measured individually; the two were combined here simply for presentation purposes.

Nano-ID[®] NPS500 Nanoparticle Spectrometer

N°O10a-5

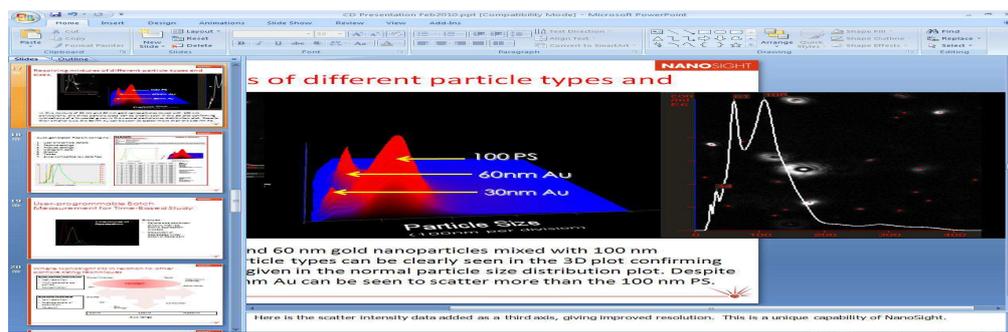
NANOPARTICLE TRACKING ANALYSIS (NTA): A TOOL IN TOXICOLOGY AND ENVIRONMENTAL FATE ASSESSMENT OF NANOMATERIAL

Phil Vincent, Patrick Hole, Pierre Peotta, Sonja Capracotta, Bob Carr
NanoSight Ltd., Minton Park, Amesbury, UK

NanoSight's Nanoparticle Tracking Analysis (NTA) is a technique for characterising nanoparticles in its native suspension. This technique provides real time and multi-parameter characterisation of nanoparticles (down to 10-20nm diameter) achieved by video analysis of a laser illuminated sample through conventional microscopy. Analysis of the Brownian motion of individual particles in the defined field of view allows their size to be determined on an individual basis. This data is then combined such that each sample is analysed for i) size; ii) high resolution particle size distribution; iii) light scattering properties (refractive index); iv) fluorescence allowing phenotyping or speciation; v) zeta potential through the application of electric fields and, vi) concentration (particles per millilitre) of any given size class of nanomaterial.

As a particle-by-particle approach NTA provides number-based size distributions that are not intensity weighted and thus reflect the true nature of environmental samples which are frequently polydisperse.

As such NTA has been applied to the analysis of a range of materials including metals, metal oxides, organic and polymer particles. NTA is especially well suited to toxicity and ecotoxicity studies and has been widely used in assessments of toxicity and the environmental fate and behaviour of nanomaterials¹. This application/area has ranged from pre-exposure characterisation for studies in direct toxicity, monitoring the colloidal stability of nanomaterial within a range of aquatic and terrestrial compartments and the impact these environments will have on particle uptake and toxicity.



Trimodal measurement of a mixture of 30 and 60nm gold and 100nm latex polystyrene particles.

¹<http://www.nanosight.com/publications/third-party-papers/-toxicity-and-environment>, or follow links.

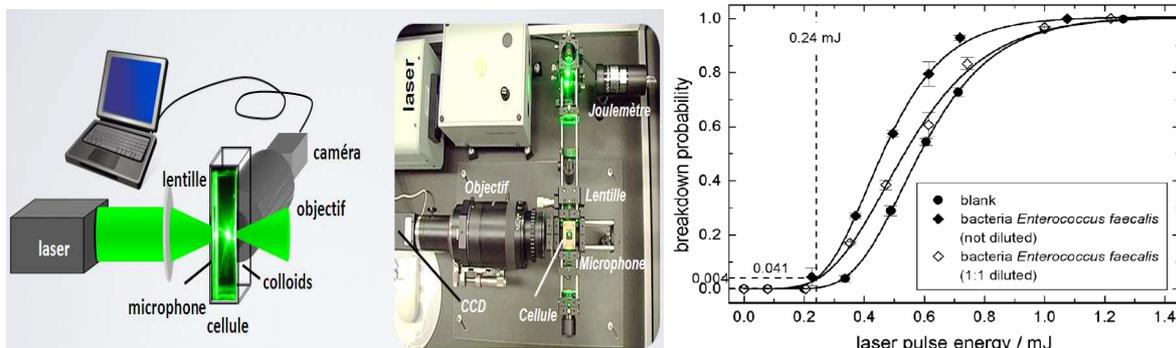
N°O10a-6

MAGELLAN: INNOVATIVE DETECTION, TRACEABILITY AND CHARACTERIZATION OF NANOPARTICLES TRACES IN LIQUID MEDIA

Stéphane Aït Oumeghar, Patrice Nagtegaele, David Jacob: Cordouan Technologies, cite de la photonique, 11 Avenue de Canteranne 33600 PESSAC/ FRANCE

The increasing use of engineered nanoparticles (ENP) in industry and in our everyday life applications raises the risk of an uncontrolled release of such substances into the environment and in particular into drinkable water resources. Moreover, for sanitary and hygienic reason removal of biological nanoparticles like virus or bacteria in drinking water is also of primary importance. To prevent such risk of contamination, it is becoming very important to water suppliers to know whether such ENP are present or not in their raw water resources, even at traces level, and whether such ENP have any impact on the efficiency of the water treatment process (membrane fouling for example). The efficiency of the treatments relies on the use of ultra filtration (UF) membranes. Such UF membranes are also widely used for seawater desalination by reverse osmosis process like those used in modern plants installed in the Mediterranean and Arab countries. Such plants, with filtering capacities of 100,000 m³/day and more represent major financial investments and have to be operated in a safe and economical way. Today the testing is conducted within the production facility as quality assurance and in the water works during operation. Common integrity tests applied in water works include measurements of turbidity, spiking tests or air pressure tests. These tests are able to detect defects of about 1 to 3 micrometers but do not provide any information on membranes defects with sizes below 100 nm and in particular in the range of viruses, which are about 20-30 nanometres. Also, Online UF membranes integrity monitoring techniques with sufficient sensitivity are still lacking and unfavourable modes of operation or damages are usually detected too late or not at all.

In this talk we present a new instrument called MagellanTM, which we believe is the most sensitive on line measurement system for Nanoparticles traces detection and particle size measurement in water. Designed by Cordouan Technologies, Magellan is a unique nanoparticle size analyzer based on a patented technology called Laser-Induced Breakdown Detection (LIBD) initially developed by the Karlsruhe Institute of Technologie (KIT). After a brief introduction on the physical principle of the technique, we show how it compares with existing methods in terms of performances and applications. We then illustrate the capability of LIBD on two concrete examples of industrial application: quantification of NP release in food by product packaging; trace detection of bio colloids (bacteria) in drinkable water



MAGELLAN Principle and optical layout (left) ; example of measurement curve for bacteria detection

N°PL11

RISK MANAGEMENT FOR OHS EXPERTS

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In many countries, employers have the primary responsibility for providing a safe and healthy workplace. This is achieved by managing risks which include identifying hazards, assessing exposures and risks, and implementing measures to control risks. Risk management for nanomaterials is problematic because of uncertainties about the nature of hazards, issues in exposure assessment, questions about appropriate control methods, and lack of OELs, or nano-specific regulations. In the absence of OELs specific for nanomaterials, a precautionary approach has been recommended in the US and elsewhere. The precautionary approach entails minimizing exposures by using engineering controls and PPE. Generally, risk management utilizes the hierarchy of controls. Ideally, risk management for nanomaterials should be part of a facility-wide risk management program. In addition to risk control, a risk management program should include a medical surveillance program that assesses the frequency of adverse effects among groups of workers exposed to nanomaterials. In some cases, the medical surveillance should include medical monitoring of individual workers. All medical surveillance should be used to assess the effectiveness of risk management.

N°O11a-1

HORIZON-SCANNING AND IDENTIFICATION OF EMERGING RISKS AMONG NANOTECH-COMPANIES

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In order to understand how companies identify and handle emerging risks related to nanomaterials, we completed more than 15 in-depth interviews with occupational health and safety (OHS) representatives in various Danish organisations. Companies varied not only greatly in regard to number of employees, overall R & D capacity and health and safety personnel, but also in regard to level of which, they already use/produce products that contain nanomaterials. Surprisingly, very little research has been done on how companies become alert to emerging issues although this initial identification of emerging risks is a prerequisite for any subsequent risk management. Key unanswered questions evolve around how companies: 1) Initially identify potentially emerging risks; 2) Collect and analyze data on these risks; 3) how they communication results of their analysis internally and externally; 4) how they complete their analysis of management options and subsequently implementation of these management options and finally, 5) what the implications of action taken are or has been. Through our interviews we found that the level of awareness about the potential occupational risk associated with nanomaterials varied greatly and range from “having just heard about potential risks and planning to look into it” to “having initiated an internal full risk assessment currently under external peer-review”. Employees noting “that something might not be right” as well as media rumors turned out to be the two main sources of identification of emerging risks, whereas ad hoc personal and non-formal networks and meetings with academics and health care officials also played a role in larger organisations. Various sources were used to obtain more factual information including: Google; Newsletters from the National Research Centre for the Working Environment in Denmark; chemistry databases and scientific articles, but the information gathering process itself was somewhat unsystematic and seems to be completed ad hoc over time. Internal data analysis was performed by occupational health personal within the companies in constant consideration of resources available, priorities, possible management options, etc. unless individual OHS representative “felt like it” putting far more effort into it. Very often independent external experts were consulted in order to learn more and get outside confirmation of key internal findings and interpretations of the available literature. The selection of management options often involved a pro et con analysis of various options considering various technical and operational barriers whereas implementations often followed a process of: 1) Double-checking that company is in compliance with existing legislation and guidelines - often non-NM specific; 2) Initial mapping of NM R & D within the company; 3) Initiation of a capacity building process; 4) Mapping of NM exposure (sometimes very extensive); 5) Mapping of health effects among employees (again sometimes very extensive) and 6) Mapping and implementation of possible management options. Overall, very few options had been implemented about mostly “easy”, “low-hanging fruit” - PE-options and administrative controls. Limited or no implementation of more thorough process-related options or engineering controls was identified primarily explained by the continued uncertainty related to health effects of nanomatierals as well as uncertainty about legislation and best practices. Results were mostly communicated internally via intranets or internal workshops with health representatives. Externally communications included publication of scientific papers, posting of information on company websites, but surprisingly involved no or very limited dialogue with authorities. This was considered irrelevant at best and potentially a source of additional confusion and bureaucracy. Overall, implications of action taken within the companies have had little impacts, but in general company representatives noted that they had gotten an improved knowledge about NM risks; that there was a general alertness regarding risks related to airborne NPs and – in some companies - that there was an increased dialogue between workers and leadership.

N°O11a-2

RISK ASSESSMENT OF NANOMATERIALS AND NANOPRODUCTS – ADAPTATION OF TRADITIONAL APPROACHES

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The new technological developments of materials at a small size scale with novel physicochemical properties offers enormous prospects for industrial applications like cosmetics, food packaging, clothing, surface coating, and paints. At the same time, an increasing number of workers and consumers are exposed to a broad range of nanomaterials and potentially faced to new adverse effects. Questions have been raised whether the current risk assessment paradigm that have been designed for 'conventional materials and products' would be applicable and adequate for the new materials, products and applications of nanotechnology.

Different approaches have been adopted in the EU for assessing the risks for human health from exposure to conventional chemicals and products. Triggers for risk assessment are in general the quantities of a chemical, the properties like persistence or bioaccumulation and the effects like acute and chronic toxicity, sensitization, genotoxicity, reproductive toxicity or carcinogenicity. In general, the traditional approach is a chemical-by-chemical approach, focusing on a single media, a single source and a single toxic endpoint. Scope and responsibilities for the development and implementation of a risk assessment concept for a concrete risk management context like chemicals authorization or consumer products safety vary across sectors and areas and depends on the specific regulatory environment. Thus, risk assessment implication is a complex task based not only on science based knowledge but also on the regulatory context involving different parties and stakeholders.

Chemical risk assessment falls under the Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and the detailed guidance documents provided by the European Chemicals Agency (ECHA). It is characterized by a four stage algorithm, involving hazard identification, dose-response assessment, exposure assessment and risk characterization. Products risk assessment falls under the sector specific regulations like the approaches for foods, novel foods, cosmetics and biocidal products considering the matrix in which it is delivered to the end-user.

A 'nanospecific' risk assessment goes beyond present standard paradigms and strategies for conventional chemicals and products. Challenges are the definition and identification of nanomaterials in a regulatory context and the limited and inconsistent knowledge database. This knowledge gap includes also general methodological uncertainties like measurement and dose-describing strategies, appropriate rules for the prediction of the behavior in biological systems and ecosystems and the question of the sensitivity of current test methodologies to nanospecific effects. SCENIHR evaluated the current methods of risk assessment and concluded that the methods are in principle applicable to nanomaterials, but specific aspects require further development. The presentation gives an overview of possible incremental approaches and additional tools for a nanospecific risk assessment:

- adaptation of current test methodologies and introduction of default values and assessment factors for data gaps,
- possible assessment of the uncertainty by weighing the data and expression of uncertainties for the purpose of transparency, consistency and scientific quality,
- comparative risk assessments in a tiered approach comparing the nanoproduct with the conventional product,
- combining risk assessment with the lifecycle methodology especially for nanoproducts,
- combining expert risk assessment with risk management for the development of risk-based classification systems (German NanoKommission, Swiss Precautionary Matrix).

Strength and weakness of the different complementary tools and their support for decision-making under uncertainty will be discussed.

N°O11a-3

OHB BASED RISK ASSESSMENT METHOD FOR POWDERS AND NANOMATERIALS

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The current risk assessment methods on nanomaterials had been experienced over the past four years and had not been found suitable at both research and industrial scale. In 2011, the RIP oN 3 of REACH had encouraged the development and the validation of new control banding tools. In this context, our approach is based on the method of the OHB (Occupational Hazard Band) widely spread in the chemical industry, and used for many years within the L'Oréal Group. Our objective was to supplement the pre-existent methods with a tool suitable for assessing the risks inherent to powders, including nanomaterials, but we did not want to divert from them for reasons of coherence and of homogeneity.

Sanofi and Ineris have been associated to our thinking process in order to consolidate our approach and we are currently testing the resulting method with other companies to fine tune the risk assessment process and to make it widely applicable in the industry.

We chose not to modify the existing scales of toxicity because the international discussions are still ongoing on this subject. We take the European classification and CLP for point of departure of our reflections and we concentrate on the control of the exposure that is the only thing on which we can really act.

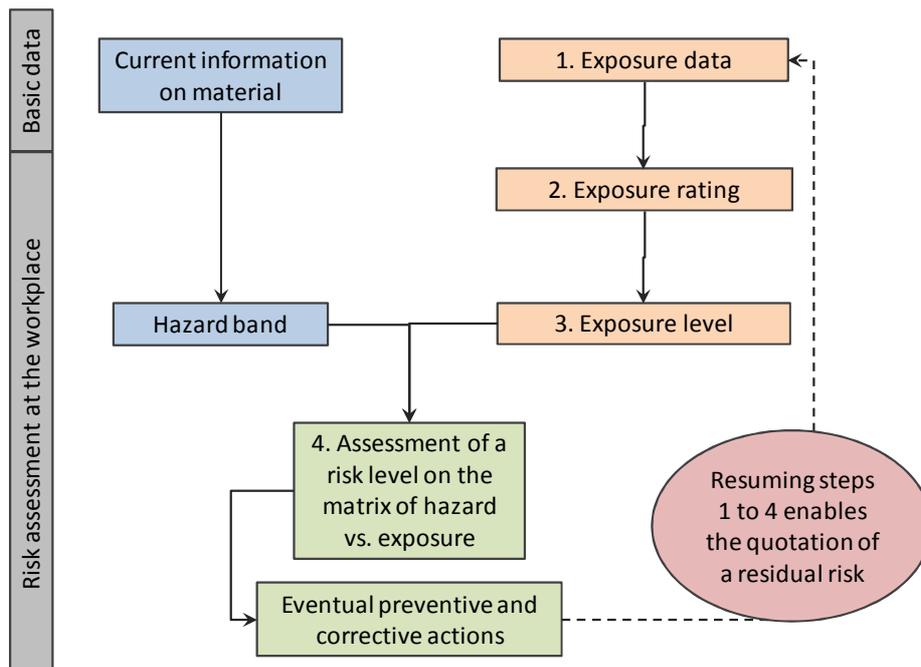
The method applies essentially to the prevention of the exposures by airborne materials, whatever their particle size. The skin exposure is not treated there specifically for the time being.

The method considers exposure based on seven parameters to take into account the characteristics of the materials used, their emission potential, the conditions of use, as well as classic parameters of the characterization of the exposure as the duration and the frequency.

The method stresses on a pragmatic exploitation of the current knowledge and of the available data, bearing in mind that a lot of them are not easily accessible to plant operators.

The product of the reflection is then positioned on a hazard x exposure matrix from which 3 levels of priority of action are defined, as in the classical OHB method applied to pure chemical risk.

This approach enables us to fill in a gap in term of risk assessment and to avoid to jeopardizing all that had been set up for years, while introducing new elements of reflection accessible to all operators without exception, in a way that the operational and the industrial hygienists can ask the right questions before figuring out the safety at the workplace.



N° O11a-4

FRENCH APPROACH FOR CHARACTERIZING POTENTIAL EMISSIONS AND EXPOSURE TO AEROSOLS RELEASED FROM NANOMATERIALS IN WORKPLACE OPERATIONS.

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There are a number of occupational situations where potential for emission and exposure to nanomaterials exist along the complete life cycle of the nanomaterials, i.e. research, synthesis, downstream use, application or treatment of products, and waste recycling/disposal.

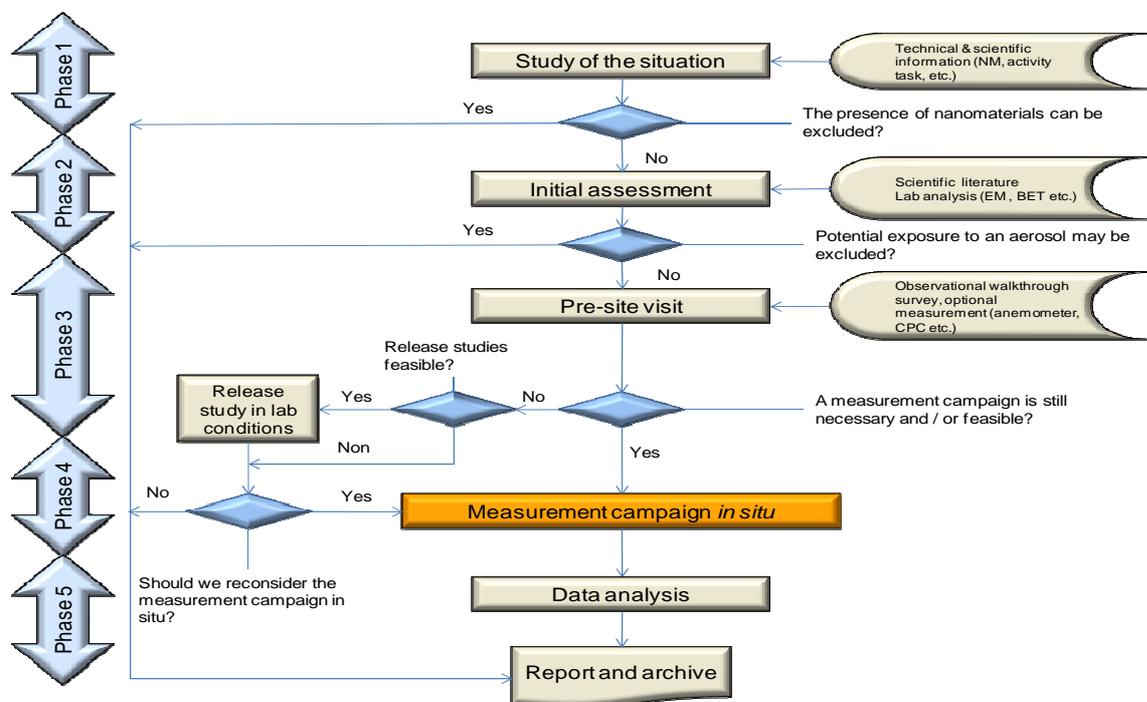
While research continues to address questions of nanomaterials toxicity, there is a need to gain a better understanding of actual workplace exposures, practices, and also determine the effectiveness of existing control measures in reducing exposures that are in place. These field studies are of particular importance as workers, whose number is growing, are the first people in society that are exposed.

Various strategies have been proposed worldwide to assess workplace exposure (BSI, 2010; Consent German Report, 2011; Methner et al. 2010; Ramachandran et al., 2011), but so far, no definite standardized international method is available.

In that context, a working group was established in 2010 in France with CEA, INRS and INERIS (CEA and INERIS being involved in the French action plan NanoINNOV), whose the outcome is guidance for the characterization of emissions and inhalation exposures to aerosols where nanomaterials are processed or used during workplace operations.

The tiered-type approach that will be presented follows a 5-stage scheme (Figure 1). Three levels should be successively passed before a measurement campaign is considered. The measurement strategy in itself is based on two levels of intervention: level 1 is intended primarily for industrial hygiene practitioners, while level 2 is intended primarily for experts / researchers with experience in (nano) aerosol measurement in the workplaces.

In addition to the international proposals, the present approach enables to integrate the possibility of particle release tests under laboratory conditions.



Given the emerging nature of the knowledge on the risks associated with nanomaterials, this approach will require revision leading ultimately to a harmonized approach at international level.

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N°O11a-5

CONCERNS RELATED TO SAFETY MANAGEMENT OF ENGINEERED NANOMATERIALS

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Recent efforts aim at developing strategies for initial assessment of the risks encountered with engineering nanomaterials (ENPs) use without requiring detailed knowledge on their toxicity. In control banding approach [1], applied to nanomaterials, Hazard and Exposure bands are estimated and combined into broad risk classes. Different levels of protection are proposed consequently to control the risk. In recent years a few teams [2-5] proposed qualitative risk estimate and control for ENPs based on this approach and it seems to be, at the moment, a largely accepted strategy to handle 'unknowns' in management of Nanomaterials safety.

We proposed [6] an approach similar to control banding to handle ENPs safety in research environment. The method consists of a schematic decision tree allowing classifying laboratories handling or producing ENPs into three hazard classes (from Nano 3 - highest hazard to Nano 1 - lowest hazard). Protective measures are subsequently proposed for each of these classes. The idea behind making the methodology simple is to allow a rapid determination of the precautionary hazard class by users of nanomaterials themselves.

In this work we will discuss practical implementation of the above-cited approach at the Ecole Polytechnique Fédérale de Lausanne in Switzerland.

The preliminary inquiry told us that: there are about 30 processes that classify laboratory into Nano 3 type (over 70 in Nano 1 type). The main processes types are: use of different types of nanopowders for producing new materials, production and use of nanofibers and a multitude of processes including ENPs in suspensions. The most frequently used types of particles are: TiO₂, Au, SiO₂, Carbon nanotubes, Fe₂O₃, Ag, RuO₂, Fe₃O₄, ZnO etc.

Results of analysis obtained for selected processes will be compared with the ones obtained using other published methodologies in order to have an objective vision in managing ENMs safety in research environment.

In the absence of dose-response relationships and exposure data, quantitative risk assessment is currently not possible for worker inhalation exposure to ENPs. Since these data are missing for most of the situations, we deduce that the result of analysis for a particular process is essentially the same with the proposed methodology [6] and the one taking into account the hazardousness of ENPs. It is evident that when reliable data on hazardousness factors (as surface chemistry, solubility, carcinogenicity, toxicity etc.) will be available, they will be integrated in the decision tree accordingly. On the protective measures side, more differences are revealed between the outcomes of different methodologies; some of them proposing very comprehensive measures and others remain with basic ones. The implementation and control of protective measures, problematic of high interest for occupational safety and health specialists, will also be discussed.

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N°O11a-6

**DESIGN OF A PREVENTION APPROACH: FROM REPRESENTATIONS TO ACTION
THE CASE OF NANOMATERIALS**

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The widespread use of nanomaterials in all sectors represents important scientific and economic issues but is also accompanied by uncertainties about their effects on health and the environment. In this context both a source of innovation and uncertainties, some research or industrial laboratories experience difficulties in implementing measures to prevent risks associated with nanomaterials. Unable to wait for the construction and stabilization of toxicological knowledge, it seems important to implement prevention in companies taking into account the actual work activity.

This intervention is part of a research program "Building and managing risks associated with nanomaterials in industry and research laboratories", whose objective is to explore ways of managing this emerging risk. This multidisciplinary research device combines prevention specialists (chemistry, metrology) to researchers in the social sciences (sociology, ergonomics, management) through the partnership of two different laboratories: the National Institute of Research and Safety (INRS) and PACTE laboratory (University of Grenoble).

This paper describes the ergonomic intervention carried out in an industrial company whose activity is focused on the development of agricultural materials rich in starch for the production of such papers. This company, especially the Research, Development and Industrialization Department, wants to test a product in nanostructured form but is faced with refusal of employees handling this product. The implemented methodology combines interviews with real work observations in reference situations and has two objectives: understand the representations of nanomaterials risks and create a space for collective debate on the professional practices within the framework of the current and future working activity.

This area of regulation has led to many changes at once:

Technical: certain practices have evolved through the use of new equipment (volumetric flask, plastic pots, largest mixing pot mixing)

Organizational: the identification of a higher risk activity during the preparation of the slurry has led to the development of a space dedicated to handling the nanostructured product and to the separation of office spaces from handling spaces.

And social by the mobilization and employees participation to the structuring of a prevention approach.

Beyond the measurement of employee exposure, it is to focus on the daily work activity and representations of nanomaterials risks among different actors mobilized in order to help them to anticipate the future working situations and to socially build an approach of collective and effective prevention. These first results show how preventive action and the work activity of employees may be resources for innovation in the enterprise.

N°O11a-7

FROM NANOMATERIALS RISK PERCEPTION TO RISK MANAGEMENT: LAST LITERATURE SURVEY LESSONS

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No risk management can be effective without accurate risk evaluation and risk perception. In the workplace, face to nanomaterials with their differences and uncertainties about health and safety effects, what are workers and stakeholders risk representations and how hazards are specified and measured ? A recent literature survey was posted on June 2012 by European Agency for Safety and Health at Work (EU-OSHA) which summarize the general principles and theories of risk perception and communication and current trends and guidance on these topics. As author in INRS, we propose to tell review main findings.

The information in the EU-OSHA literature survey is drawn from searches of the published literature, Internet and grey literature, studies that have been done on the perception and communication of the risks of nanomaterials and initiatives or practices noted down in EU countries.

The review found “serious gaps in awareness of the potential risk involved in handling nanomaterials at work and serious shortcomings in the way that the risks are communicated to workplaces“. Also, report consider the gap in knowledge and limitations of the risk communications initiatives and suggest ways these might be addressed. Today, a large fraction of the public still knows very little about nanotechnology and nanomaterials : even if there are large differences between North and South countries, in Europe a majority of people has never heard of nanotechnology (EC, Eurobarometer, 2010). Despite to this low level of knowledge, the public expects benefits to predominate over risks for nanotechnology. These results contradict the usual findings, particularly the fact that when risk objects are „new“, „unknown to science“ or „not observable“, they are generally judged as highly risky. However there is a growing concern about risks to workers. And experts are more concerned than the public with regard to pollution and „new health problem“ of nanomaterials (contrary to situation with usual risks). Businesses' major issue of concern is not occupational hazard but the existence of obstacles to innovation. In businesses, concerns also vary with activity (producer vs user), experience in handling materials and the size of companies (SMEs are less informed about risks). And expectation that benefit judgment will increase with knowledge should be treated with caution. Even if we think that risk acceptance increases with knowledge (Kahan et al., 2009), risk judgment seems to be highly malleable and can move in either direction (Satterfield et al, 2009).

In managing nanomaterials risks, it is important to be aware of the generally limited knowledge of the public and that understanding and perceptions differ significantly between countries, organisations and workplaces. People, including workers, are very easily influenced by their social group's shared values, particularly in relation to technology and the environment. An assessment of worker's attitude and likely behaviour should be included in workplace risk assessment and management.

N°O11a-8

**FRENCH REGISTRY OF WORKERS HANDLING ENGINEERED NANOMATERIALS AS
AN INSTRUMENT OF INTEGRATED SYSTEM FOR SURVEILLANCE AND RESEARCH**

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During the last decade important body of toxicological and exposure data was generated in the framework of occupational safety and health (OSH) research focusing engineered nanomaterials (ENM). However the lack of epidemiological and biomedical data on the human health potential risks related to the ENM exposure is a substantial gap for validating experimental results, risk-assessment modeling and developing consistent risk management guidelines if needed. Considering that despite these uncertainties, ENM handling spreads in industry, the French government officially charged the InVS to develop an epidemiological surveillance of workers occupationally exposed to ENM.

Our objectives were to conceive an operational plan of such surveillance and to prepare all necessary conditions for its actual implementation on the national level.

An initial surveillance plan was proposed within a multidisciplinary working group held by the French Institute for Public Health Research (IReSP) on the basis of literature review and discussions with national and international ENM and OSH experts. In site investigations and technical visits (N=8) were then carried out to assess the plan feasibility and improve its effectiveness. In parallel, national required ethical approval applications as well as inter-institutional partnership agreements were prepared in view of further technical and scientific cooperation.

The current plan consists of a multi-step methodology where exposure registry construction is paramount. Two ENM were chosen as priority for this registry: carbon nanotubes and nanometric titanium dioxide considering available toxicological data, quantities manufactured in France and projected for production development, the choice of France in the framework of the sponsorship program for the testing of ENM sponsored by OECD, and social perception factors. Workers potentially exposed to these ENM will be identified using a 3-level approach: 1-identification and selection of companies concerned with ENM exposure (based on compulsory declaration and questionnaires), 2-in site exposure assessment and identification of the jobs/tasks with ENM exposure (based on job-exposure matrix construction, further supplemented with measurements), and 3-identification of workers concerned. Workers identified will be included in the registry, with data of interest collected by questionnaire. Companies and workers inclusion questionnaires are designed and currently under validation. This registration is at the moment planned for three years but can be longer extended and include other ENM. A prospective cohort study will be established from this registry, to pursue surveillance objectives and serve as an infrastructure for performing epidemiological and panel studies with specific research objectives.

Conclusion: The protocol of the registry construction is now operational. The obtaining of all necessary approvals including that of the French authority in charge of privacy and personal data protection is expected by December 2012. The registration of first potentially exposed workers should start since January 2013. Initially programmed for three years, this registry should be considered as an instrument of integrated system for epidemiological surveillance and national and international OSH research in the field of ENM. This will be able to provide early data concerning human health potential risks associated with ENM in France.

N°PL12a

REGULATORY ASPECTS OF NANOMATERIALS IN REACH

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The Commission Communication on the Second Regulatory Review on Nanomaterials underline the potential of nanotechnology to create major technological breakthroughs, growth and jobs. At the same time, the Commission sets out the plans to improve EU legislation to ensure the safe use of nanomaterials.

The Review's main conclusions: Nanomaterials are similar to normal chemicals/substances

- In the light of current knowledge and opinions of the EU Scientific and Advisory Committees and independent risk assessors, nanomaterials are similar to normal chemicals/substances in that some may be toxic and some may not. Important challenges relate primarily to establishing validated methods and instrumentation for detection, characterization, and analysis, completing information on nanomaterial hazards and developing methods to assess exposure to nanomaterials:
- Possible risks are related to specific nanomaterials and specific uses. Therefore, nanomaterials require a risk assessment, which should be performed on a case-by-case basis, using pertinent information. Current risk assessment methods are applicable, even if work on particular aspects of risk assessment is still required.
- Overall the Commission remains convinced that the Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) Regulation is the best possible framework for the risk management of nanomaterials when they occur as substances or mixtures, but within this framework more specific requirements for nanomaterials have proven necessary. The Commission envisages modifications in some of the REACH Annexes and encourages ECHA to further develop guidance for registrations after 2013.
- In order to improve the availability of information on nanomaterials, the Commission will create a web platform with references to all relevant information sources, including registries on a national or sector level (where they exist). In parallel, the Commission will launch an impact assessment to identify and develop the best means to increase transparency and ensure regulatory oversight, including an in-depth analysis of consequent data gathering needs. This analysis will include those nanomaterials currently falling outside existing notification, registration or authorisation schemes.

The presentation in the conference will provide more insights on regulatory aspects of nanomaterials in REACH.

N°O12a-1

**LEGAL ISSUES OF THE ENVIRONMENTAL SAFETY REGULATION IN THE SPHERE OF
NANOTECHNOLOGY IN RUSSIAN FEDERATION**

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Nowadays one of the principal innovative spheres in Russia is considered to be a rapid development of nanotechnology and nanomaterials and implementation its products into almost all fields of social and industrial life. Nevertheless, despite the pointed advantages an appearance of nano products has set not only a positive example of the scientific progress development and dynamics, but also a potential threat of possible risks for the environment and people's health in the case of its massive use when there is a lack of research about its impacts. In Russian Federation the problem of nanosafety regulation is extremely actual. A number of international documents have noted a lack of legal regulation in the sphere of nanotechnologies in Russian Federation. Thus, according to the strategic EU program, it's mentioned that in 2009 on an annual meeting of OECD Tour de Table Meeting in Paris (November, 2007) Russia took responsibility to develop a long term program of nanoindustry development by 2015 (Nanotechnology Action Plan for Russia-2015). The key role should be dedicated to the nanosafety aspects and potential risk assessment of nanomaterials for environment and humans' health. Unfortunately, until now Russia did not provide this document for discussion at the international level by the partners of nanoconsortium. Indeed, from 2007 until nowadays in Russian Federation there are no a single federal act of legislation (federal'nyi zakon), establishing the state foundations of regulation of nanosafety. The only one Federeal Legal Act in the described sphere is the Federalniy Zakon from 19 July 2007 «About the Russian corporation of nanotechnologies», which has established the principles of organization, activites, functions and termination of Rosnanotech. However, there are no articles dedicated to the environmental safety provision in regards of nano products applications. Also there is no complex federal act consolidating legal status of action in the field of development and application of nanoproducts, inspite of fact, that these relationships have a significant financial segment. Thus, according to the Federal Act from December 13, 2010 «About federal budget for 2011 year and the period of 2012 and 2013 years" the expenses for realisation of program «The infrastructure development of nano industry in Russian Federation for 2008-2011» were 250 milliard of Russian rubbles. The profound analyses of the current legal systems has demonstrated that nowadays the main legal regulation in nano sphere consist of some range of *frame documents* (accentuated by the author). Thereby, one of the most dynamic and developed field of innovative activities in Russian Federation – nanotechnology - is left out of environmental and legal area of protection and that is might lead to the raising level of ecological risks at the stage of creation and application nano products to the environment and humans' health. During the analyses of annual norms of the Report OECD « Nano technologies: the Environment, health and safety» the conclusion is following – Russia has an extremely low degree of conceptual realization of the program in the sphere of forming the policy connected with nanotechnology and its impact on the environment and also a lack of implementations of norms into the national legal systems in terms of the assessment criteria of nano safety EHS (Environmental, Health and Safety) and ELSI (Ethical, Legal and Social Issues). To the great regret, there is no independent and precise legal act about the ways to creat and apply nano products with the certain definitions and principles and, more importantly, with the level of legal obligations and responsibilty. This gap is not possible to fill by just altering and editing the existed legal acts due to the lack of the state Russian regulation. Thus, one of the most dynamic fields of innovative activities – nano technologies – is practically out of the regulation. It might lead to an increase of ecological risks' level in the process of creation and application of nano products to the environment and health of people. As a result, implementation of international legal recommendations in the field of safety regulation of nanotechnology is quite crucial for Russian Federation.

N°O12a-2

SAFETY IMPLEMENTATION OF NANOTECHNOLOGY FOR CHEMICAL ENTERPRISES IN THE CENTRAL EUROPE SPACE

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Introduction: The general objective of the Central Europe project NANOFORCE, which is developed by national and regional chemistry associations and R&D Centres of the Central Europe area, is to foster the innovative nanotechnology-sector networks across Central Europe regions by bringing together public and private organizations to carry out collaborative and interdisciplinary researches on nanomaterials (in the frame of REACH Regulation) and to turn the most promising laboratory results into innovative industrial applications.

To build up a legal advisory board for chemical enterprises starting in nanotechnology, a state of the art report on existing safety procedures and nanotech related regulations was produced to give an overview on currently available regulations used by chemical industries and manufacturing companies within the European region to secure their products. The main emphasis was placed on REACH regulation to search for relevant sections concentrating on nanomaterials which are applicable for nanotechnology. In addition, all relevant directives and amendments of REACH were screened with regard to identify gaps where action is still needed and give possible recommendations for the European Commission. Beyond literature research a questionnaire for producers, users, researchers and financiers was developed with the goal to collect information about the nanotechnology sector in the CE region concerning development, financial status, international cooperation within joint ventures, safety and nanotoxicology.

The BioNanoNet Forschungsgesellschaft mbH is an Austrian network which combines a wide range of expertise in numerous disciplines of medical and pharmaceutical research in nanomedicine and nanotoxicology and acts as work package leader in the NANOFORCE project. Additionally the BioNanoNet functions as coordinator of the European Center for Nanotoxicology (EURO-NanoTox), an Austrian example of a European hub in nanotoxicology. Within the aim of EURO-NanoTox, the BioNanoNet offers regulatory expertise, dissemination and communication service, scientific reviewing of testing methods and a panel of standardized in vitro and in vivo toxicity tests, with the overall goal of driving innovative interdisciplinary research.

Focus: As result of the gradually increasing nanotechnology sector there is the necessity of a contemporary analysis of the present regulations used for nanomaterials, to outline the current situation of the nanotechnology sector, to promote international cooperation and research's coordination to overcome disciplinary boundaries, to fill the gap between more and less experienced regions and to turn investments in R&D in industrial innovations.

Within this contribution existing regulations for nanomaterials (in the frame of REACH) used within the European region as well as first results of the NANOFORCE project concerning safety implementations will be presented. Furthermore the BioNanoNet will present its work and the EURO-NanoTox as well as the ONLINE-Journal "EURO-NanoTox-Letters".

N°O12a-3

**DEFINING OCCUPATIONAL AND CONSUMER EXPOSURE LIMITS FOR NANOMATERIALS -
FIRST EXPERIENCES FROM REACH REGISTRATIONS**

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By 1 December 2010 substances produced and imported ≥ 1000 t had to be registered under REACH Regulation. JRC carried out an assessment of what type of information on nanomaterials was provided in received registrations (Nanosupport project¹). On this basis, JRC examined the adequacy of REACH requirements for nanomaterials and developed options for possible specific nanomaterial provisions building on the current REACH framework.

From 26000 submitted registration dossiers covering 4700 substances finally 21 dossiers were considered to cover nanomaterials or nanoforms of a substance. These dossiers were subject to a detailed analysis and assessment of information provided for all endpoints including substance identity, physico-chemical properties, human health, fate, ecotoxicity, PBT assessment, Classification and labelling as well as the Chemical Safety Report.

In order to evaluate how the the safety of workers and consumers was ensured, it was appropriate to check how the "Derived No (minimum) effect Levels" (DN(M)ELs) were established for substances, (potentially) covering nanomaterials. DNELs were established mainly for long term inhalation exposure of workers. In one dossier DNELs were derived for short and long term exposure for all exposure routes and for workers, consumers and the general public. Half of the assessed dossiers included an oral long term DNEL for the general population. DNELs were usually not specific for nanosized forms and, in the few cases where they were calculated for nanosized materials, they were not derived from hazard data for the nanoform.

Different methods for deriving the DNEL were applied. Several DNELs were based on available OELs for inhalable and respirable dust or the nuisance dust levels, which have not been established for nanosized materials. Few dossiers derived DNELs by applying the default assessment factors in the REACH guidance. In general lower (i.e. less strict) assessment factors were applied with different types of justification. All DNELs were expressed in mass/volume metrics.

It is important to note that submission, identification and selection of the dossiers addressed in this study was done before the adoption of the EC recommendation (2011/696/EU)² on a definition of nanomaterial and before the publication of the revised ECHA guidance documents that include recommendations for nanomaterials³. Further, it must be stressed that the project did not assess the compliance of the dossiers analysed, but focused on how the REACH regulation could be adapted to ensure a proper information generation and reporting and an appropriate risk/safety assessment of nanomaterials.

This presentation will cover the most relevant results from the Nanosupport project and provide an overview on available guidance for defining occupational and consumer exposure limits for nanomaterials.

¹ http://ec.europa.eu/environment/chemicals/nanotech/pdf/jrc_report.pdf

² <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:275:0038:0040:EN:PDF>

³ http://echa.europa.eu/web/guest/view-article/-/journal_content/3df5b7b9-a36d-4e74-811b-3aeed23366f8

N°O12a-4

**DEVELOPMENT OF AN INTEGRATIVE PROGRAM OF NANOSAFETY: PROMOTE THE
COORDINATION BETWEEN INDUSTRIES AND RISK ASSESSOR.**

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Nanotechnology is an emerging science involving manipulation of matter at the nanometer-scale level. The recent rise surrounding nanotechnology stems from its potential to revolutionize diverse area. Nanomaterials are widely present in many industrial sectors (chemical, biomedical, environment, etc.) and their application is expected to expand significantly in coming years. This growth of nanomaterial raises many questions regarding the potential risks to human health and the environment and more specifically the occupational health. Indeed, the available literature supports the ability of the lung, gastrointestinal tract, and skin to act as a significant barrier against systemic exposure for many nanomaterials. However, in front of a potential risk issue related to the toxicity of nanoparticles to the biological material, tools need to be developed for improving the risk management of the regulators. The goal is to develop a tool which examines the current knowledge base regarding the health risks posed by engineered nanoparticles, in order to improve nanotechnology safety prior to marketing phase. The approach proposed in this work is based on the establishment of a safety assessment approach constructed on a decisional control pathway regarding nanomaterial production and consumer's product integrating different aspects such as: (i) primarily research and identification of nanomaterial base of physicochemical properties, toxicity and application, (ii) the occupational exposure risk during the manufacturing process, (iii) and the engineered nonmaterial upon the consumer product. This approach provides important parameters to reduce the uncertainty related to the production of nanomaterials prior their commercialization, reduce the reluctance from the industrial, and provide a certification tool of sanitary control for the regulators. This work provides a better understanding of a critical issue of nanomaterials and consumer safety. It also enables significant to improve the future planning regulated to risk assessment of nanomaterials and should improve the acceptability across the population.

Funding: *Special thanks to the NE3LS, IRSST and BioSimulation Consulting Inc for funding this work.*

Keywords: *Nanotechnology, nanomaterials, safety assessment, social impact.*

N°PL12b

NANOSTAIR PROJECT: ESTABLISHING A PROCESS AND A PLATFORM TO SUPPORT STANDARDIZATION FOR NANOTECHNOLOGIES

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Standardization is one of the most adequate solutions to quickly capitalize and disseminate knowledge and have it implemented in the industry to ensure sustainable competitiveness, according to “reference documents”. It is very important in the field of nanotechnologies since the production of knowledge is very intensive.

The overall objective of nanoSTAIR project is to build a sustainable process and platform in the field of nanotechnologies to support the transfer of knowledge gained through research to standard documents, in the context of the STAIR approach promoted by CEN & CENELEC.

The project is organized around several activities that will boost the development of new standards.

First the project will set-up a mechanism to identify, with a bottom-up approach, the opportunities for standardization from the results of European research projects, co-funded by the European Commission but also co-funded by National Research Programmes. This mechanism will be established using existing networks and initiatives such as NanoSafety Cluster or NanoFutures, as well as the network of the national standardization bodies and their partners in the various Member States. Then, resources and consortia sharing similar standardization opportunities will be pooled together to launch standardization work items. The last supporting activity consists in providing assistance to select the right standardization umbrella (Technical Committee and Working Group at CEN or ISO level) and launch new standardization work items.

nanoSTAIR will provide a set of procedures and a tool box that will bridge the gaps between research and standardization in nanotechnologies. It will structure and ease the development of new standards, and thus enable the European nanotechnology related industry to rapidly operate according to the state of the art.

The presentation during the conference will give an overview of the nanoSTAIR initiative and the first developments.

List of participants:

Participant no.	Participant legal name	Country
1.	 EU-VRi - European Virtual Institute for Integrated Risk Management	Germany
2.	 Institut National de l'Environnement Industriel et des Risques	France
3.	 Finnish Institute of Occupational Health	Finland
4.	 Tecnalia Research & Innovation Foundation	Spain
5.	 Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek	Netherlands
6.	 Steinbeis Advanced Risk Technologies GmbH	Germany
7.	 DIN Deutsches Institut für Normung e. V.	Germany
8.	 European Committee for Standardization	Belgium
9.	Jean-Marc Aublant – Chair of CEN TC352 Nanotechnologies	France
10.	Michael Stintz – Member of ISO 229	Germany

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N°O12b-1

**CURRENT INTERNATIONAL STANDARDIZATION IN PARTICLE CHARACTERIZATION AND
RELEASE TESTING**

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The interdisciplinary field of particle and particle release characterization requires coordinated actions between the measurement methods standardizing ("vertically" oriented) Technical Committees like ISO/TC 24/SC 4 "Particle Characterization" and application scenarios standardizing committees ("horizontally" oriented) like ISO TC 229 and CEN TC 352 "Nanotechnologies" or ISO/TC 256 "Pigments, dyestuffs and extenders". Liaisons, represented by experts, between these committees have to ensure the detailed coordination in the daily work and for instance the mandate M/461 to CEN, CENELEC and ETSI for standardization activities regarding nanotechnologies and nanomaterials asks for managing the coordination. The following example illustrates the current interaction of such standardization activities.

Within ISO/TC 229/WG 2 the Project Group 10 has developed a general framework for determining nano-objects release from powdered engineered nanomaterials into gaseous surrounding by means of the analysis of the generated aerosol particles (ISO/TS 12025: 2012 "Nanotechnologies — Quantification of nano-object release from powders by generation of aerosols"). This framework supports also future standardisation of nanocomposites testing, because for instance defined abrasion procedure generate in an intermediate step wear powder before dispersing it. Figure 1 shows an example for a downscaled process for the quantification of the released particle number from surface coatings during sanding, which is the subject of a new standardization project in ISO TC 256.

Basic components of the differential mobility analysing system for aerosol particle size classification were in the focus of standardization in ISO/TC 24/SC 4/ WG 12, which recently released the ISO 15900: 2009 standard "Determination of particle size distribution — Differential electrical mobility analysis for aerosol particles". Currently WG 12 has circulated a Committee Draft ISO TC/24 SC/4 N623, ISO CD 27891 "Aerosol particle number concentration - Calibration of condensation particle number counters"(CPC). Both together allow the online aerosol particle characterization in the test methods, standardized by the "horizontal" committees above.

"Calibration" of the CPC has been done using a Faraday-cup aerosol electrometer (FCAE) as reference standard in the overlapping particles size and concentration range according to Figure 2. In many cases, the purpose of the 'calibration' was to determine the limit of particle detection at very small size. The FCAE has been used as the reference since the detection efficiency of the FCAE was considered to be unity at any size. The detection efficiency of a CPC is determined as the ratio of the concentration indicated by the CPC under calibration to that by the FCAE, while aerosols of singly-charged, size-classified particles of the same number concentration are supplied simultaneously to both instruments.

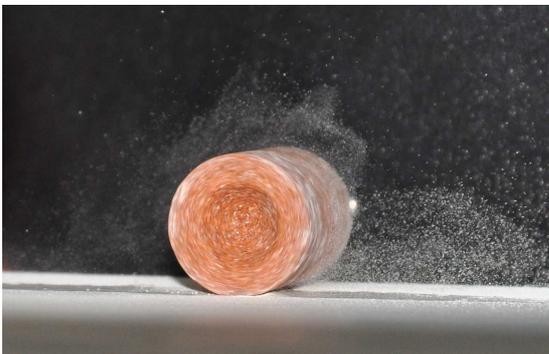


Fig. 1: Test for the release of particles from surface coatings during sanding, quantification of the released particle number

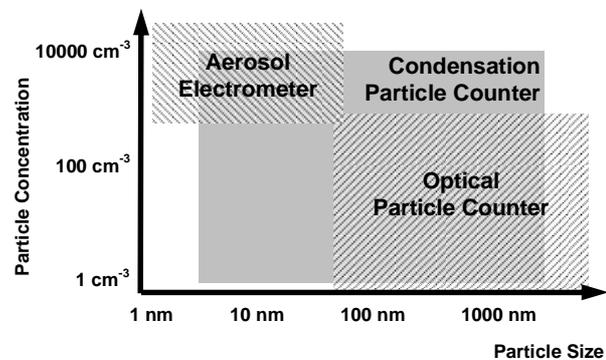


Fig. 2: Concept of particle number concentration standard (detection efficiency determination) in ISO/TC 24/SC 4/ WG 12

N°O12b-2

**OVERVIEW OF STANDARDIZATION ACTIVITIES IN EUROPE (CEN)
AND IN RELATION WITH ISO AND OECD**

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

One of the building block of the “safe, integrated and responsible” approach is standardization. Both the Economic and Social Committee and the European Parliament have highlighted the importance to be attached to standardization as a means to accompany the introduction on the market of nanotechnologies and nanomaterials, and a means to facilitate the implementation of regulation.

In the beginning of 2010, EC DG Enterprise and Industry addressed the mandate M/461 to CEN, CENELEC and ETSI for standardization activities regarding nanotechnologies and nanomaterials. Thus CEN TC 352 “Nanotechnologies” has been asked to take the leadership for the coordination in the execution of M/461 and to contact relevant international and European Technical committees and interested stakeholders as appropriate. Prior requests from M/461 deal with Characterization of and exposure from nanomaterials and any matters related to Health, Safety and Environment.

N°O12b-3

MANAGING OPERATION PROCEDURES IN COLLABORATIVE PROJECTS

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The EU-funded FP7 project NanoDiaRA, whose aim is to develop nanoparticles and biomarkers in early diagnosis of inflammatory diseases, requires transparency and reproducibility of the scientific and technological outcome in the different research fields which are connected through 15 partners. By establishing standardized operation procedures (SOPs) following a clear composition and using a specifically developed tool for structured storage and exchange of results and protocols (“Electronic Sample Book”), such requirements can be achieved.

Innovative projects today – being at the different levels of research and development at small or large scale – are characterized by an increasing technical complexity and often physical distance between the project partners. The expertise of the partners often comes from a variety of technological areas. To gather comparable, reproducible results, a proper documentation of the used methods is crucial.

The two main steps in assuring such a documentation are establishing proper protocols in the form of SOPs and providing access to these protocols to every project partner as well as a clear allocation of SOPs to conducted measurements, production steps, etc.

An SOP is a protected document that should be common to a lab/partner in order to reproduce similar functions (i.e. experiment how to prepare samples, exploit data, prepare administrative documents, and send samples). SOPs are usually implemented fulfilling GLP and GMP requirements to respect quality standards. The overall procedure should be established in agreement with all users. Some of the challenges that have to be overcome are:

- The document should contain enough information so that anyone using it can repeat it, but must be concise enough for clarity of the reading.
- A way must be found that it can be adapted to different situations/needs (give options: e.g. “for urine proceed...”; “for organs proceed...”).
- SOPs must be distinct and unique: only one version at a time should exist; access has to be controlled. Rules for upgrading have to be established.
- It must be clear which version of the protocol was used for earlier experiments. This can be done by a versioning system.
- Limits must be set: definition of a range of acceptance.
- Protocols must be accessible for every project partner.

In our talk, we like to outline a new strategy for the handling of scientific operation procedures involving the methods and applications we developed, and make suggestions for SOP management in collaborative projects in general.

Title	Sample Type	Description	Author
Zeta Potential measurement I	Nanoparticle	Zeta potential measurement using photon correlation spectroscopy, Test process Documents: ESB_Test_protocol_09.pdf	WP1
Uptake, MTS and Cells counting	Biological Sample	Test process Documents: ESB_Test_protocol_06.pdf	Test group IV
Turbidity measurement	Nanoparticle	Turbidity measurement by UV-VIS spectrometer, Test process Documents: ESB_Test_protocol_12.pdf	Test group II
SPION synthesis	Nanoparticle	Synthesis of 400 ml of Superparamagnetic Iron Oxide particles Documents: ESB_Test_protocol_22.pdf	Test group I

Figure 3 : Structured SOP storage in the Electronic Sample Book.

N°O13b-1

**TRIMATEC COMPETITIVENESS CLUSTER
MANAGEMENT OF CONTROLLED ENVIRONMENT FOR NANO FIELDS**

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Created in 2005, Trimatec competitiveness cluster has two main objectives:

- participation in the development of innovative ecotechnology projects
- promotion of the attractiveness and expertise of the regions

Trimatec's strategy consists in establishing and developing structured ecosystems in each of the Cluster's four thematic areas, one of which is management of confined environments.

Control of these environments is a response to the need to protect individuals, the environment and products. The technologies developed find applications in industries such as pharmaceuticals, medical research, micro-nanotechnology, nuclear field, microelectronics.

Technologies used in industries such as pharmaceuticals and nuclear fields could bring interesting solutions for the nano fields.

Trimatec, with the companies involved in confined environments area, will present examples of these



N°O13b-2

TREATMENT OF LIQUIDE EFFLUENTS CONTAINING NANOMATÉRIALS

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The treatments of effluents containing nanomaterials or/and nanoparticles aim at modifying the size of the particles compatible with a conventionnal treatment allowing to eliminate from the primary effluents or through specific stage in the classical treatment steps. The processes used for the liquid/solid separation are flocculation/filtration/flottation.

N°O13b-3

**SAFE ECODESIGN AND SUSTAINABLE RESEARCH APPLIED TO NANOMATERIAL
DEVELOPMENT (SERENADE CONSORTIUM)**

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Nanomaterials (NMs) are expected to be a key in innovation breakthroughs and to lead to many new applications by 2020 (Roco 2011; Roco, et al. 2011b). However it is also well accepted that Nanomaterials will be transferred from the research laboratories to industry and finally to the end-consumer, only if issues on societal challenges such as **sustainability and acceptance** will be perfectly taken into account. Considerable attention has now to be paid by companies to develop a sustainable approach by incorporating safety consideration from the **design stage and during** the entire **production of the new generation of NMs**. To reach this goal, the laboratories and companies consortium SERENADE aims at creating a new approach in order to develop tomorrow's nanomaterials, safer for both humans and environment.

N°O13b-4

**SYNTHESIS OF ACTIVE TiO₂ NANOPOWDERS AND THIN LAYERS
USING SUPERCRITICAL CARBON DIOXIDE PROCESSES
APPLICATION TO EFFLUENT TREATMENT**

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percritical carbon dioxide (SC-CO₂) based processes are attractive for ceramic materials preparation and were initially developed in our group for the synthesis of oxide ceramic nanopowders [1-5]. During the last 15 years, we investigated a wide range of materials (TiO₂, ZrO₂, Y-ZrO₂, CeO₂, Gd-CeO₂, SiO₂...) and applications (photo-catalysis, catalysis, solid oxide fuel cells, gas purification, cosmetics...). Prepared powders present highly interesting characteristics such as high specific surface areas, nanosized crystallites and multi-scale structures that can be controlled by changing process parameters (pressure, temperature, stirring...).

The presentation will focus on nanophased TiO₂ synthesis, characterization (structure/morphology) and properties (radionuclide adsorption/photo-catalysis). A special care will be held on the direct processing of these TiO₂ nanoparticles in supercritical CO₂ to prepare photo-catalytic contactor or TiO₂/substrate composites for effluent treatment. Compared to TiO₂ obtained from various methods (sol-gel, CVD, Degussa...), TiO₂ powders and contactors synthesised in supercritical CO₂ has shown excellent results in terms of photocatalytic activity under UV beam, notably demonstrated for organic dye-molecules decomposition. Especially, direct supercritical CO₂ deposition of TiO₂ on porous inorganic substrates highly improved particles properties, decreasing nanocrystallite aggregation and increasing accessible active surface. In addition, first results on Sr²⁺ adsorption using these nanometric TiO₂ powders seem quite promising for nuclear effluent treatment.

Key words: Supercritical CO₂, photo-catalysis, sorption, effluent treatment, TiO₂

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[5] S. Sarrade, L. Schrive, C. Guizard and A. Julbe: *Manufacture of single or mixed metal oxides or silicon oxide, Patent WO9851613 (1998)*

N°O13b-5

EXAMPLES OF SYSTEM ENGINEERING FOR NANOPARTICLES HANDLING AND STUDIES.

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2 systems are presented to illustrate engineering work dedicated to nanoparticles.

The first one is an hybrid confinement system that is configured by user safety needs. This system called mini-PSPN is a safety hood that can be transformed into a glove-box within 10 seconds without any adjustment. A state of the art qualification test is done for both configurations. This solution provides a new adjustable tool for laboratories working with various materials in a limited space.

The second system is a test chamber build with the intention to characterize nanoparticles emissions from equipments in domestic and professional environments. A preliminary design phase using a numerical model has been carried out. It allows us to integrate requirements on sampling representativeness, on particle residence time and on the temporal resolution coming from the anticipated emissions characteristics. The results we obtained during the performance tests indicate that this test chamber can measure emissions in every location of the test space with a sampling error of nearly 15%.

N°O13b-6

**PERSONAL PROTECTIVE EQUIPMENT AGAINST NANOPARTICLE
SELECTION EVALUATION AND FUTURE STANDARD TO BE BUILT**

Samuel Ozil¹, HONEYWELL PROTECTIVE CLOTHING, ZA de Berret 30200 Bagnols :Ceze

As Nanomaterials (NMs) are expected to become present in our daily life, we have to consider how to protect the workers those have to face the generated risk at the industrial phase. Manufacturing, incorporation,... up to the waste treatment.

What are the doors of access to the organism; how to reduce this risk by the appropriated means. How to evaluate the efficiency of the PPE; against the ultrafine particle. Is there any lesson that can be taken from our experience related to the contamination prevention against solid pollutant simulated by the $1/3 \mu$ NaCl.

What are the problems to face when comes the time to build a standard relative to the PPE evaluation against nanosized particle...

N°P1b-1

OCCUPATIONAL EXPOSURE ASSESSMENT FACILITY AT VITO

Evelien Frijns, Inge Nelissen, Patrick Berghmans, VITO NV (Flemish Institute for Technological Research), Boeretang 200, 2400 Mol, Belgium

The QNano Transnational Access (TA) element is dedicated providing users from the European nanosafety community access to nanomaterials processing, characterisation and exposure assessment facilities. Proposals for access may be submitted in response to calls. The calls and the application forms are available online (<http://www.qnano-ri.eu/>).

VITO Belgium offers their occupational exposure assessment facility for Transnational Access which includes:

- Instrumentation to determine particle concentration (number, surface area, charge, sampling) and particle size in air;
- Specific nano aerosol generators;
- An indoor aerosol test chamber.

Because field testing is difficult to arrange, expensive and usually uncontrolled, the instrumentation and test chamber can be used to:

- simulate processes (abrasion, wear and tear) and activities (nano powder handling) emitting nanoparticles;
- generate nano aerosols and study aerosol physics (e.g. dispersion, agglomeration);
- identify processes likely to produce highest occupational exposures
- characterize temporal and spatial variations of aerosol concentrations at specific nano processing
- evaluate performance of real-life engineering controls of nano aerosol
- (inter)compare instrumentation;
- optimize measurement methodologies and sampling techniques.

Specifications aerosol test chamber:

- Size: 6 (l) x 4 (w) x 3 (h) = 72 m³
- Negative pressure system (safety)
- Controllable flows (inlet/outlet/recirculation)
- HEPA filtered inlet (particle free) also adaptable to unfiltered inlet (background)
- HEPA filtered exhaust
- A panel perpendicular to the flow for injecting generated aerosol
- Panels for guiding through sample inlet tubes
- Possibility to split up into two rooms through movable panels with flow circulation
- Temperature and humidity monitoring and logging

N°P1b2

RISK ASSESSMENT OF NANOMATERIALS

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New technologies require risk assessment process early in their development stage. Many expert reports have been published in regards to risk-assessment procedures, which considering their general principles, offer a particular framework for assessment of nanotechnology risk. Additionally, the risk-assessment should include ways in which nano particles come into contact (through inhalation, skin contact, and oral application).

Moreover, each of the proposed techniques for risk-assessment poses constrictions in nano risk-assessment in terms of calculating the determining components of risk and its occurrence possibility which must be taken into account. Before applying nano materials to everyday products, they should be first tested for their probable toxicity, compatibility and harmlessness from bio aspects while their probable hazardous impact must be evaluated before being applied widely. Due to increasing number of consumer goods and industries enjoying nano-materials, human beings will be more at risk of nano-posed hazards and such materials can turn to a major health threat for human community and ecosystem. The following essay compiles the result of analytical studies in risk assessment as well as health and safety of ecosystem issues concerning nanomaterials.

Keywords: Nanotechnology, Risk assessment, Health, Safety, Environment, Toxicity.

N°P1c-1

**APPLICATION OF CURRENT AVAILABLE MODELS FOR THE EXPOSURE ASSESSMENT OF
NANOPARTICLES FROM CONSUMER PRODUCTS**

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Consumers are exposed directly and indirectly to chemicals as a result of their contact with everyday consumer products. Currently, there are hundreds of consumer products on the European market which contain engineered nanoparticles, including food packages, personal care and cosmetics products, sunscreens, disinfectants, and clothing.

However, there exists a very limited understanding for human exposure to nanoparticles from such products and resulting health effects. The apprehension about exposure to nanoparticles is based on the fact that their chemical and physical properties may differ substantially from the properties of the same materials in bulk.

Because of the huge amount of consumer products and usage, the exposure routes can be very different including the intentional and unintentional uptake. Nanoparticles can be basically absorbed above the three fundamental routes 'oral', 'dermal' and 'inhalative' even during intended use.

Inhalation is assumed as a critical uptake route and may be the key exposure route to human body. Therefore, sprays might be considered to be critical to human health because engineered nanoparticles can attain to the pulmonary region of the respiratory tract due to their small size.

Our goal was to verify the applicability of published data for exposure assessment via sprays containing estimated amounts of different engineered nanoparticles with computational tools currently applied in the regulatory contexts.

ECETOC TRA and ConsExpo are suitable programs with mathematical models for assessing the potential exposure of consumers to chemicals from consumer products. The question is to what extent these models are appropriate for the application of exposure assessment to nanoparticles from consumer products. Which parameters have to be changed or included in these models to make them applicable.

In contrast to the more consumer-oriented models, there are two further models to assess the exposure of nanoparticles: Stoffenmanager Nano and MPPD. These models include specific properties of nanoparticles such as particle size and shape. In our investigation, we evaluate the possible assignment of these models for the exposure assessment of nanoparticles from consumer products and discuss the possibility of using the banding approach, which is normally used in occupational exposure assessment, as well in the field of consumer exposure assessment.

N°P1c-2

**DEVELOPMENT OF A NANO EXPOSURE AND CONTEXTUAL INFORMATION DATABASE
(NECID)**

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For future research in studying exposure to nanoparticles, there is a need for an (occupational) exposure database, regarding measurements of airborne particles made in field studies that can be considered nanoparticles. Amongst a working group of PEROSH institutes, IFA and TNO are developing a database structure called NECID (Nano Exposure and Contextual Information Database), which will include exposure data and contextual information. The database will be developed to collate a substantial amount of nano exposure data from a diversity of locations and exposure scenarios. The database will facilitate the future comparing and sharing of nano exposure data, because the exposure data of different research institutes are collected and stored in a uniform and harmonized way. The new nano exposure database will be based on the characteristics of existing databases (ART database, MEGA) and the NANOSH dataset. As nanomaterials have distinctive characteristics and the measurement strategy is based on a multimetric approach, additional many variables should be introduced. The proposed structure of the future database covers a set of contextual core information variables in the database, which should be collected with each set of measurements based on Rajan et al. (1997) and Tielemans et al. (2002). Terms of use should be composed for research institutes and third parties. Different user-specific rights may be awarded for the entering, reading, reporting and export of data for different users. The development of NECID is currently in progress and a first beta version of NECID is expected by the end of 2012.

N°P2a-1

EFFECTIVE AND SELECTIVE EXTRACTION OF SILVER NANOPARTICLES FROM ENVIRONMENTAL WATER BY AN IONIC EXCHANGE RESIN

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The production and use of engineered nanomaterials such as silver nanoparticles (Ag-NPs) continues to increase rapidly, but their fate and impact in the environment largely remain undetermined. In particular, there is little information on the concentration of nanoparticles (NPs) in real and complex environments, which has been a critical bottleneck hindering in-depth investigation of their potential risk. Recent studies, operated via various models, have predicted that the concentration of Ag-NPs in environmental water would reach 80 ng L⁻¹ within the next 10 years. Even so, this may not necessarily indicate that the quantification of NPs such as Ag-NPs in environmental samples can be ignored.

To date, established analytical tools for NPs quantification have focused so far on synthetic products and are in the most cases not suitable for complex mixtures, like environmental or biological samples, nor are they able to detect very low concentrations (ng L⁻¹). Therefore, as a first step sample preparation, i.e. extraction and preconcentration of NPs from complex matrices is required. Herein, we report an effective and selective extraction of Ag-NPs from environmental water by an ionic exchange resin.

An anionic exchange resin containing positively charged ammonium groups was used in this study. Moreover, Ag-NPs coated with four different surfactants such as citrate, PVP10, PVP40 and TOAB were tested by this proposed method. The surfactants-coated Ag-NPs were modified by a bifunctional ligand containing carboxyl and thiol groups before the NPs pass through a column with resin. The bifunctional ligand can anchor the NPs on the surface of resin via electrostatic force. Comparison of the Ag concentration before and after passing through the column with resin, shows that more than 89% of Ag-NPs are loaded onto the surface of the resin. Afterwards, more than 85% of the citrate-, PVP10- and TOAB-coated Ag-NPs can be eluted by 8% formic acid in methanol. However, a significantly lower recovery was observed with Ag-NPs coated by PVP40. Beside effective extraction the most important issue for this novel method is the preservation of size and shape of the Ag-NPs. On the basis of TEM analysis, we confirm that no significant changes in particle size and shape occur.

In environmental waters Ag-NPs will be stabilised by interaction with natural ligands, such as dissolved organic matter (DOM). In this respect, the presence of DOM in the Ag-NPs solution might be critical for the proposed extraction method. We found that the DOM at a concentration of 2 mg L⁻¹ in the initial Ag-NPs hydrosol had no effect on the separation and extraction of Ag-NPs. Furthermore, in order to provide a method for preconcentration of M-NPs from natural aqueous samples especially separation from metal ions is required. Only then element selective detection methods are applicable for the quantification of metals present as M-NPs. We found that the recovery of Ag-NPs was more than 96.4%, while less than 10% of the Ag ions were recovered, indicating that the proposed method has the potential to selectively extract and preconcentrate NPs even from an aqueous mixture containing the NPs and corresponding metal ions.

To further evaluate the applicability of the proposed method for real natural waters, three different real environmental water samples were tested by spiking 80 ng L⁻¹ Ag-NPs. The obtained recoveries of Ag-NPs are all higher than 69.1%, which is satisfactory with regard to the low spiking level. Moreover, the corresponding enrichment factors of Ag-NPs are higher than 138.

In conclusion, we present a new method to effectively and selectively extract Ag-NPs at ng L⁻¹ level from real environmental water like river, lake and brook. In addition, preservation of size and shape of these NPs after application of the method was proved by TEM analysis of the concentrated NPs suspension. Hence, application and optimisation of this method to a broader variety of water matrices and for extraction and preconcentration of other M-NPs from environmental waters are currently under investigation.

N°P2a-2

CNT-DETECT: PERSONAL SAMPLER AND CORRESPONDING PORTABLE READING DEVICE

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At present, carbon nanotubes (CNT) in air can only be detected by deposition on a substrate and the use of off-line imaging analysis such as SEM. The majority of techniques suitable for the quasi-real-time measurement of engineered nanoparticles (ENP) such as electrical low pressure impactor (ELPI), scanning mobility particle sizer (SMPS), and condensation particle counter (CPC) can not distinguish between nanoparticles in general and airborne nanofibres in particular. In common workplace settings the considerable background of fine and ultrafine particles thus poses a challenge for these instruments.

Furthermore, a true portability of the present devices is not given due to their size and power consumption. However, there is an urgent need due to the expected toxicity of nanofibres to control workplace environments with a robust and mobile device to ensure the safety of the working personnel as soon as possible.

To meet these challenges, a personal sampler is developed. This portable personal sampler collects CNTs on a specific magnetic substrate. The collected CNTs can be analyzed quasi-online with a portable Raman-based optical detection device which will give a qualitative and quantitative result based on the collection duration and flow rate of the sampler. With Raman detection, it was even possible to distinguish between CNT sources from different manufactures. Sensitivity and detection limit are currently under investigation and will be shown at NANOSAFE 2012. The magnetic sampler can also be used for the collection of other airborne magnetic particulate matter. A special-designed substrate for automated off-line REM-EDX analysis is presented. The automated REM-EDX-analysis enables counting, size and material classification of the collected airborne magnetic particulate matter.



Personal sampler for airborne magnetic particulate matter

N°P2a-3

APPLICATION OF ENZYME IMMUNOASSAY FOR FULLERENE C₆₀ DETECTION IN RATS' ORGANS

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An increasing growth of manufacturing fullerenes and their wide application for industrial and biomedical purposes causes a need for new approaches to control fullerenes in living organisms and the environment. In comparison to HPLC, which now serves as a main technique for the detection of fullerenes, immunochemical assay can be considered as a promising alternative. This method has such advantages as rapidity, high sensitivity and possibility to analyze a row of probes simultaneously. However, significant difficulties can appear when developing immunoassay of fullerenes. Carbon nanoparticles (CNP) with their unique structure are unconventional immunogens, which do not have inducers of immune response typical for natural biopolymers. In addition, obstacles to solubilization of hydrophobic CNP in aqueous media are expected to be additional problems in immunoassay realization.

Until now, little research has been done on the development of immunochemical analysis of fullerenes. In this study, we developed the enzyme-linked immunosorbent assay (ELISA) for the detection in the first use of free fullerenes C₆₀ and C₇₀ and multi-walled carbon nanotubes in solutions and in organs and tissues of animals administered with fullerenes.

The immunization of rabbits and mice by the conjugate of fullerene C₆₀ carboxylic derivative with thyroglobulin synthesized by carbodiimide activation led to the production of anti-fullerene polyclonal antisera and monoclonal antibodies, correspondingly. The specificity of the antibody–fullerene binding was confirmed. The interaction of the produced antibodies with fullerene was characterized by the registration of surface plasmon resonance changes in Biacore system. The equilibrium association constants measured by this technique varies from 1.6 to 66 10⁶ M⁻¹. The developed indirect competitive ELISA allows to detect not only water-soluble fullerene derivatives (such as protein-conjugated fullerene, fullerene aminocaproic acid and fullerenol), but also pristine fullerenes C₆₀ and C₇₀ and multi-walled carbon nanotubes in solutions. To solubilize extremely hydrophobic fullerenes a specially selected dimethylformamide – phosphate buffer mixture compatible with immunoassay was proposed. The detection limit of the developed ELISA varies from 0.04 to 5.5 ng/ml for different CNP forms; the duration of the assay is 2 h. To confirm the specificity of antibody–fullerene interaction a homogeneous fluorescence polarization immunoassay (PFIA) was applied as the alternative approach. The detection limit of fullerene C₆₀ by PFIA is 1.2 ng/ml; the assay duration shortens to 10 min.

Fullerene C₆₀ was detected by the developed ELISA in homogenates of rats' organs after intraperitoneal or intragastric administration. To reduce the influence of biomatrix on the assay results a technique for the sample pretreatment was developed. It is based on the extraction of C₆₀ by toluene followed by the evaporation of toluene and dissolution of the fullerene-containing residue in the above-mentioned water-organic media.

After single intraperitoneal administration (250 mg/kg) fullerene was detected in liver, spleen, kidneys, lungs and brain of rats. Fullerene C₆₀ was also found in liver, brain, and intestine of rats at days 7, 18 and 30 of chronic intragastrical administration with different doses (0.5-50 mg/kg). Thus, the immunoassay procedure in the first use allows the detection of fullerene C₆₀ in animals' organs and tissues.

The obtained results open opportunities for applying the produced antibodies and the developed assay to toxicological studies, environmental monitoring and technological control of the CNP production.

The work was supported by the Federal Target Program «Development of nanoindustry infrastructure in the Russian Federation in 2008–2011».

N°P2a-4

NEW PERSPECTIVES IN MANUFACTURED NANOPARTICLES CHARACTERISATION: OVER THE SIZE LIMITS

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The need of physico-chemical characterisation nanoparticles is today of great concern. One of the major challenges is their size and electrical charge determination. More especially the capability to obtain manufactured nanoparticles length distribution is crucial in order to improve and control the manufacturing process chain and final quality of the products (ex: Carbon NanoTubes CNT).

The use of an on-line separation technique hyphenated to several detector(s) is intrinsically capable to take up this characterisation challenge. More precisely, Field-Flow Fractionation (FFF)-based couplings including Ultra Violet (UV) and Multi-Angle Light Scattering (MALS) have been demonstrated to be relevant and reliable analytical tools to achieve a multidimensional particle characterisation *i.e.* to obtain complementary physico-chemical information by jointly using complementary techniques. This is because FFF, associated to a convenient sample preparation, offers the capability to separate manufactured nanoparticles and characterise them. It is also because MALS intrinsically gives a size measurement with no need of calibration, which guaranties the reliability of the results.

Asymmetrical Flow FFF (A4F) hyphenated to MALS has been validated as giving an accurate length distribution from single and multi-walled CNT samples (SWCNT and MWCNT) over the nanoscale and up to the microscale, with a repeatability not exceeding 5% in length [1]. A4F-UV-MALS has been also showed to be a relevant technique for nanotube dispersion monitoring [2, 3]. More particularly, it is capable to determine the presence or absence of bundles/aggregates, number and size of different populations and their corresponding size distributions. From this coupling it is also possible to differentiate various CNT productions according to their post- synthesis treatment. Complementary, Cyclical-Electrical FFF (CyEIFFF) can be used to reach the electrophoretic mobility (mean and distribution) of different CNT samples, as it was recently showed for different types of SWCNT [4]. More generally this technique allows separating CNT according to their charge based-properties and distinguishing various types and various nanotube productions.

Finally, through the different original examples that we proposed to present, the analytical power of FFF-based techniques to characterise manufactured nanoparticles, monitor them according to their original state including state of dispersion and give relevant qualitative and quantitative can be highlighted.

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N°P2a-5

**QUANTITATION OF CARBON NANOTUBES ON FILTERS USING A LASER-INDUCED
BREAKDOWN SPECTROSCOPY SYSTEM. LABORATORY AND ONSITE MEASUREMENTS.**

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Laser-induced breakdown spectroscopy (LIBS) is developed to monitor the presence of nanoparticles in ambient atmosphere at workplace. The LIBS technique consists in focusing a laser pulse onto the surface of a sample, and in analyzing the emission spectrum of the resulting plasma. It is multielemental, fast and does not require any sample preparation. Therefore, it is well suited for onsite continuous measurements of hazardous compounds. For higher sensitivity, we chose to sample nanoparticles on filters and to measure by LIBS the concentration of elements of interest. Previous results obtained in our laboratory with titanium and iron oxide nanoparticles confirmed that this approach enables to reach sufficiently low detection limits, compatible with existing workplace exposure recommendations [1].

Our final purpose is the monitoring of carbon nanotubes (CNTs). In this talk we will present the different steps leading to the development of a LIBS system for airborne CNTs measurements. We first optimized the choice of the filter type. Then, the detection limit of the system was evaluated in laboratory, well controlled conditions. Finally, the system was used on the production site of a CNTs manufacturer to detect a potential release of CNTs at different places of the production workshop. No significant release of nanoparticles was detected during this measurement campaign.

Performances, limitations and challenges of LIBS analysis for airborne nanoparticles monitoring will be discussed.

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N°P2a-6

**DIFFERENTIATED ANTHROPOGENIC AMBIENT PARTICLE SIZE SPECTRA WITH A
NEW U-SMPS SET-UP**

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Real time continuous and accurate measurements of atmospheric particles are important when complying with regulation or in the context of source apportionment; e.g. in determining how much is contributed by anthropogenic sources like traffic, industry, residential heating or by natural sources like dust sand or sea salt. One important characteristic of ambient air particles is the interaction with water. In general the origins of hydrophilic particles are natural sources whereas most hydrophobic particles like soot or hydrocarbon droplets are anthropogenic. By measuring this particle characteristic an important contribution to ongoing discussions regarding the source apportionment can be made.

For size dependent real-time measurements of the amount of natural and anthropogenic particles a new SMPS set-up with two CPCs is presented. This setup allows the measurement of time-resolved particle size distributions in the sub-micrometer size range with two CPCs. The two CPCs are exactly the same in regards to design and operation. The key feature of the used Palas[®] UF-CPC is that by simply changing the saturator and condenser working temperatures different working fluids can be used. By using water and butanol simultaneously, the interaction of the particles with water can be investigated as the two working fluids have different counting efficiencies for hydrophilic and hydrophobic particles. Subtracting the size dependent concentrations of the two CPCs, the amount of anthropogenic particles can be derived.

We will present measurements of a two week campaign in which we have investigated the usage of such a set-up for the characterization of ambient air particles. The measurements were performed in a suburban area. The time-resolved spectra supports interpretations regarding the composition of the aerosol and show that this set-up can be used to have more information regarding the source apportionment of the particles.

N°P2a-7

NANOPARTICLE DOSIMETER FOR EXPOSURE MEASUREMENT AND TEM SAMPLING

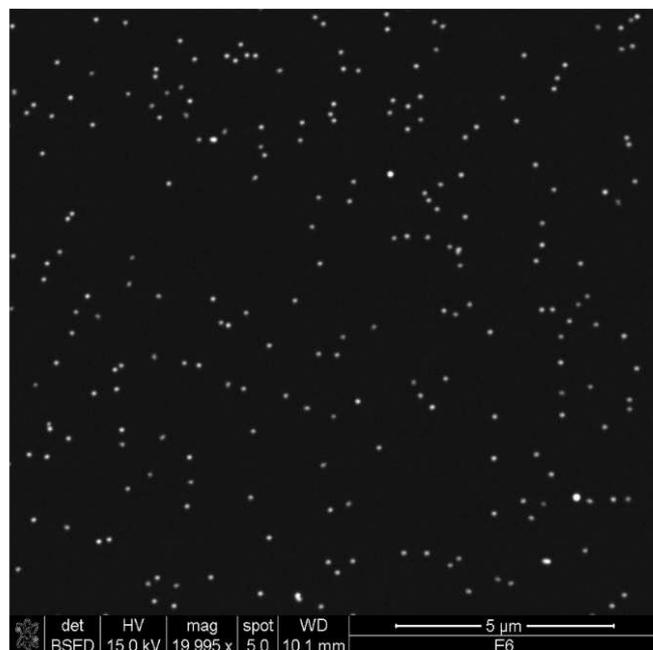
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Nanoparticles in the workplace pose a threat to health. With the increasing use of engineered nanoparticles, there is a clear need for effective protection measures. A crucial component of these is the ability to detect nanoparticle sources easily, and to measure personal exposure of workers, to ensure that standard occupational safety measures, such as encapsulation, ventilation and filtration, are working properly. Traditional particle instruments have some shortcomings for this purpose; often, they are cumbersome to use, not sensitive enough for nanoparticles, and rather expensive and complex.

One of the simplest principles of nanoparticle detection is electrical charging of the particles followed by an ultra-sensitive current measurement. This principle is realized for example in the TSI NSAM, which measures a signal which is closely proportional to the lung-deposited surface area (LDSA). The LDSA is of particular interest, as it has been shown in many studies to be more health relevant than traditional metrics such as particle mass or particle number.

We have constructed a miniature instrument to measure LDSA which can serve as a nanoparticle dosimeter. It is based on the traditional charging followed by current detection, but introduces a refinement that allows it to operate without a particle filter in the instrument. It is thus less service-intensive than instruments that require a periodic filter change. Furthermore, its operation principle also automatically compensates the electrometer zero offset.

We have also built a slightly larger version of the instrument, which includes an electrostatic precipitation section after the LDSA measurement section in which particles can be deposited directly onto a standard 3.05mm diameter transmission electron microscope (TEM) grid for further analysis in an electron microscope. This instrument thus combines a simple and robust nanoparticle detector with an optional deposition for analysis with one of the most powerful analytical techniques available for nanoparticles. Since the instrument measures concentration during TEM sampling, it can calculate the necessary deposition time for an optimal coverage of the grid.



Left: Dosimeter (134x78x29mm, 460g); right: TEM sample produced with the sampling version of the instrument.

N°P2a-8

PARTICLE SAMPLING BY TEM GRID FILTRATION

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The characterization of the physical and chemical properties of aerosols is a recurrent need in numerous studies and research projects conducted in occupational health, atmospheric chemistry and physics, stack emissions, etc.

This need is particularly felt in studies on the risk associated with nanomaterials (NM), as it is necessary to be able to distinguish particles related to NM from those of the background (urban background, other nearby sources, etc.).

Transmission electron microscopy (TEM) is among the techniques that meet this need for characterization as it allows the individual visualisation of particles and the analysis of their elementary components with an energy dispersive X-ray (EDX) probe. This is the reason that many projects are conducted for many years to enable and make more effective deposition of particles on specific TEM support products: TEM grids.

The present study evaluates the efficiency of a new particle collection technique based on filtration through one type of the TEM dedicated supports, namely TEM porous grids.

For this purpose, a universal filter holder was designed. The experimental results have been analyzed with reference to filtration theory.

The study is focused mainly on "Quantifoil 1.2/1.3" grids (Agar Scientific) carried out with a flow rate of 0.3 l.min⁻¹. Indeed, its regular structure facilitates the determination of its characteristics (pore diameter, thickness, and surface density of the pores) and consequently the use of theoretical models.

Experimental tests show that the collection is operational in the 5 nm – 150 nm size range with a minimum efficiency of 15% around 30 nm.

Simulation confirms these results and shows an increased efficiency even below 5 nm and beyond 150 nm.

The filter holder designed in this study is a low cost, portable and easy to use tool.

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N°P2a-9

AEROSOL SIZE DISTRIBUTION ESTIMATION AND ASSOCIATED UNCERTAINTY FOR MEASUREMENT WITH A SMPS

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SMPS (Scanning Mobility Particle Sizer) is a high resolution nanoparticle sizing system that has long been hailed as the researcher's choice for airborne nanoparticle size characterization for nano applications including nano research and development. SMPS is widely used as the standard method to measure airborne particle size distributions below 1 μm and it takes part in a large range of applications such as toxicology testing, indoor air quality measurement, etc. It is composed of two devices: a DMA (Differential Mobility Analyzer) selects particle sizes thanks to their electrical mobility and a CPC (Condensation Particle Counter) enlarges particles to make them detectable by common optical counters. System raw data represent the concentration of particles over several classes of mobility diameters. Common inversion procedures included in SMPS commercial software use a simplified model to ensure a fast estimation of the aerosol size distribution.

Present study aims at providing the users with an accurate estimation of the aerosol size distribution with associated uncertainty. Indeed, no dimensional uncertainty on the size distribution is provided to the users when the DMA operates under scanning mode (when the electric field is moved continuously)) while, first, it is useful for the control of the process itself and then it has been proved that ultra-fine particles can pass through human barriers (lung, blood-brain barrier, etc.).

First, improvements are brought to the model by including the effects of Brownian diffusion. Then, The sources of uncertainty arising from the measurements were determined, with a special investigation on the DMA voltage calibration and on the noise arising from the CPC counting modes (single particle count mode, live-time count mode and photometric mode). Indeed, coincidence theory failed to explain the variability on the observed raw data, so was a specific model for the counting noise needed to be developed

Inversion methods with regularization techniques proved to give more accurate results and were chosen here. Once model and inversion procedures were chosen, the statistical inversion was defined. Since Bayesian framework is well-suited for inverse problems, choice has been made to infer each component of the size distribution via MCMC (*Markov chain Monte Carlo*) methods. The final result is expressed as a confidence region on the estimated size distribution. Figure 1 shows the 95% confidence region for a bi-modal atmospheric size distribution.

Inversion and uncertainty analysis have been developed through a program coded in Matlab and can be used as an alternative to SMPS commercial software. Nevertheless, to ensure robustness and accuracy of the all process, new experiments still need to be performed.

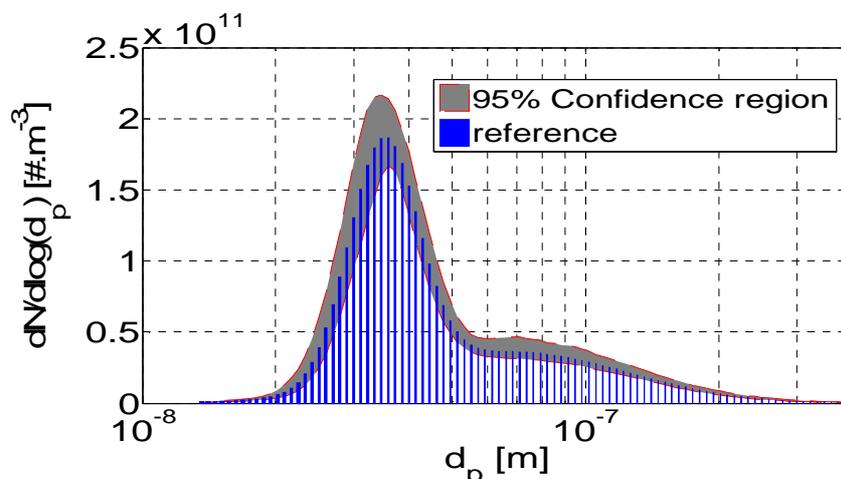


Figure 4. Estimated size distribution and its 95% confidence region

N°P2a-10

RADIOLABELLING OF ENGINEERED SILVER AND TITANIA NANOPARTICLES AS A TOOL FOR SENSITIVE DETECTION OF NANOPARTICLE RELEASE FROM SURFACE COATINGS

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Engineered nanoparticles (NPs) such as silver and titania are widely used in consumer products e.g. as functional additives in surface coatings. Due to aging or abrasion of these nanocomposites, particle release becomes likely and further transport e.g. in environmental systems could be assumed. Figure 1 shows results from a worst-case scenario study where NP release was enforced due to the use of a quite labile surface coating and photooxidative degradation of the nanocomposite catalysed by TiO₂ anatase NPs. For conventional, more stable nanocomposites, accurate and sensitive detection and quantification of NP release is still a challenge due to the fact that released fractions might be small but nevertheless significant. The application of radiotracers provides the excellent option of “visibility”, traceability and quantification of NPs in complex media. Therefore, different radiolabelling strategies for engineered nanopowders (TiO₂ P 25, Evonic Industries, d_p = 21 nm; Ag⁰, Sigma Aldrich, d_p < 100 nm) were established using a diffusive introduction of ^{110m}Ag radionuclides (t_{1/2} = 250 d) into Ag⁰-NPs (Hildebrand & Franke, 2012) and ⁴⁴Ti radionuclides (t_{1/2} = 63 a) into TiO₂-NPs through a low-temperature annealing procedure. Another radiolabelling strategy is the direct activation of Ag⁰ and TiO₂ nanopowders that was done via proton irradiation using a Scanditronix MC40 cyclotron (Abbas et al. 2010, Holzwarth et al. 2012).

Both methods were tested with respect to labelling yield, achievable activity concentration, pH-dependent stability of the labelling and the influence on NP properties. Results obtained show an appropriate selection of radiolabeling methods suitable for different experimental conditions for NP release studies.

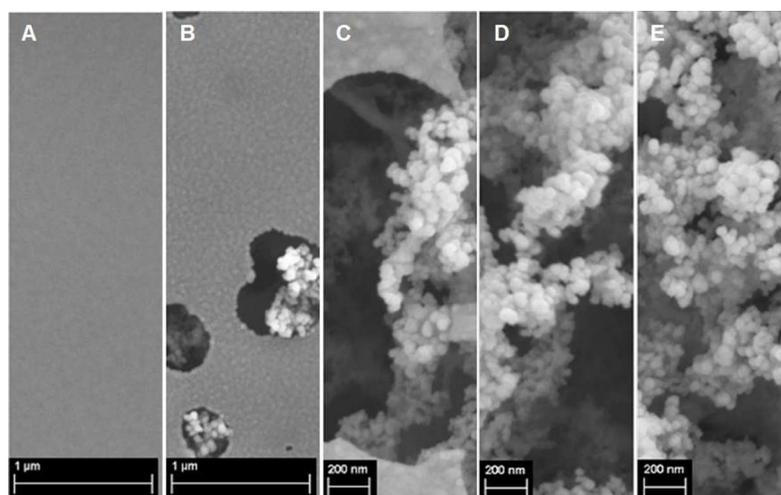


Figure 1: Time-dependent degradation of a polyacrylate TiO₂ nanocomposite by UV-A-irradiation (intensity ~ 15 mW/cm²); A) original, B) after 2 days (50.000x); C) 4 days, D) 8 days, E) 16 days (75.000x); SEM micrographs: Leibniz Institute for Surface Modification.

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N°P2a-11

**COMPARISON OF METHODOLOGIES TO MEASURE NUMBER BASED SIZE DISTRIBUTION ON
POLYDISPERSE NANOPARTICLES**

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Products containing nanomaterials are already used and their number is expecting to grow rapidly. Nevertheless, according to the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), there is still scientific uncertainty about the safety of nanomaterials in many aspects and therefore the safety assessment of the substances must be done on a case-by-case basis. In October 2011, the European Commission adopted the Recommendation on the definition of a nanomaterial (2011/696/EU). According to this Recommendation a "Nanomaterial" means: A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm. Moreover, harmonised measurement methods need to be developed to ensuring that the application of the definition leads to consistent results across materials and over time.

This definition raises the problem of measuring size distributions of nanoparticles on a number basis in a large variety of samples that can be very complex (e.g. soil, consumer product, paint etc.). This task is challenging, and the most reliable approach is to compare results obtained with different techniques. Nevertheless, different techniques are based on different principles. Therefore they measure different metrics. For instance, Dynamic Light Scattering (DLS) measure hydrodynamic diameter while Scanning Electron Microscopy (SEM) measures solid diameter. Besides, some techniques measure number based size distribution while others measure mass or intensity based size distributions. In this context, assumptions have to be made to calculate the number size distribution. Until harmonised measurement methods are available, best available methods should be applied.

The objective of this study is to evaluate available methods for measuring number based size distribution. First, we evaluated two of the mainly used methods, i.e. Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Then, Field Flow Fractionation coupled to Light Scattering and ICP-MS will also be evaluated. While DLS and SEM are widely used, it is now well recognized that they present limitation to give reliable size distribution in complex cases. For instance, when the studied nanoparticles show some polydispersity and/or nanoparticles are in a complex matrix (eg. paint, soils). In this study, the potential added value of FFF-LS-ICPMS, for those complex cases, will be evaluated. Results obtained with the three different methods will be compared for the analysis of different types of polydisperse nanoparticles (e.g. SiO₂, Ag etc.). Drawbacks of each technique will be pointed out and some analytical solutions will be proposed. The methods reliability for the analysis of real case sample (e.g. leachates containing debris of nanopaints after demolition) may also be presented.

N°P2a-12

NON DESTRUCTIVE AND FAST METHOD FOR THE DETECTION OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES (SPION) BIODISTRIBUTION BASED ON THEIR MAGNETIC PROPERTIES

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Superparamagnetic iron oxide nanoparticles (SPION) have become important for various *in vivo* and *in vitro* biomedical applications such as imaging, magnetic separation, biosensor devices and therapy. To be used in biomedical applications, SPION are usually stabilized in physiological media with biocompatible surface coating. They can also be surface modified for specific targeting or detection [1]. Analysis of biodistribution of these particles, accurate quantification of particles accumulated in organ tissues and determination intracellular iron are very important to assess nanoparticle toxicity and their targeted delivery.

Nowadays, several analytical techniques to detect SPION *in vivo* or *in vitro* exist. Magnetic resonance imaging (MRI) or magnetic resonance relaxometric methods can be used to detect magnetic particles, although MRI equipment is not available in many labs for routine use. The most widely used are chemical methods such as Induced Coupled Plasma (ICP) techniques or colorimetric assays by UV/VIS like Prussian Blue (PB) or ferrozine-based assays. By dissolving the SPION into Iron III (Fe^{III}), quantitative analyses are performed in comparison to iron calibration curves. However, even if they are commonly used these methods are destructive and not selective. It must be noted that total iron species are quantified by chemical analysis, which do not distinguish elemental iron from dissolved magnetic particles and endogenous iron in the form of iron protein complexes, i.e. in liver transferrin, ferritin or hemoglobin. Moreover the organs have to be dissolved before analysis, which could be a difficult preparation step.

We propose an easier, faster and nondestructive method to detect SPION. We use their magnetic properties to measure their magnetic susceptibility and quantify iron in nanoparticle formulation and biological samples like serum and organs. With this technique, the samples are not destroyed and can so be saved for further analyses. Moreover, this measurement is more robust to distinguish magnetic nanoparticle from tissue iron, which permit to decrease the used of control samples. More than 300 different samples were analyzed to estimate the accuracy and efficiency of this method. The influence of different coatings of SPION on magnetic measurements was also studied. It will be demonstrated that magnetic susceptibility is an ideal method requiring a minimal sample preparation, having high detection sensitivity and allowing to quantify low concentrations of particles with high reproducibility.

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N°P3a-1

SILVER DOPED HYDROXYAPATITE COMPOSITES FOR LONG-TERM BONE THERAPY

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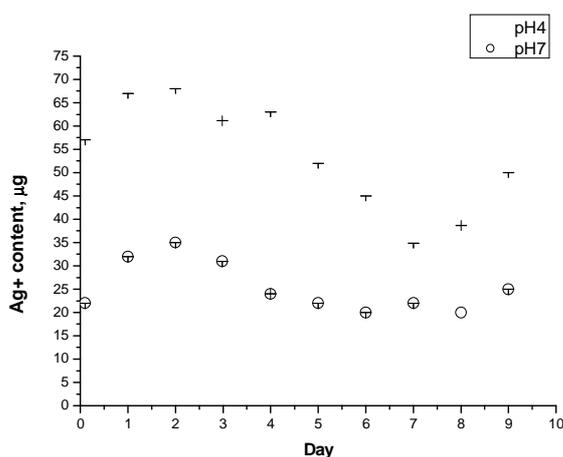
Hydroxyapatite is a soluble material that can act as a unique system for the delivery of silver ions and antibiotics in a controlled way. In our opinion, hydroxyapatite/silver/antibiotic composites are very promising as they can combine the biocompatible properties of the carrier with the antimicrobial and healing action of the additive.

The effect of doping hydroxyapatite powder (during the hydroxyapatite synthesis) with silver (0.03-0.07 wt%) on its physicochemical properties, morphology, deagglomeration, the kinetics of calcium and silver release into buffer solutions and adsorptivity towards antibiotics was investigated.

It was established that silver doping slightly affected the hydroxyapatite solubility at pH 4 and 7. The process of silver release from Ag-doped hydroxyapatite as well as calcium release took place more actively at pH 4 as compared with pH 7. It should be noticed that release of silver begins at the very beginning of experiment and lasts not less than fortnight. Therefore Ag-doped hydroxyapatite can reveal antimicrobial properties straight away after implantation. At both pH levels silver released within the amount limits sufficient for bactericidal activity and insufficient for cytotoxic effect.

Introduction of silver into hydroxyapatite markedly decreased the adsorptivity towards antibiotic rifampicin; nevertheless the process of rifampicin adsorption went on, which makes it possible to conclude that creation of a hydroxyapatite/silver/rifampicin composite is possible.

Also, the processes occurring during the interaction of Ag-free and Ag-doped hydroxyapatite with antibiotic ciprofloxacin solutions have been investigated. Particular attention was paid to the formation of a gel due to the interaction of hydroxyapatite with water/ciprofloxacin solutions. The examination of the surface morphology of the gel revealed the transformation of the morphology of both samples. The investigation into the adsorptivity of the Ag-free and Ag-doped hydroxyapatite towards ciprofloxacin showed a significant difference. The introduction of silver ions into the hydroxyapatite structure sharply reduced its adsorptivity towards ciprofloxacin. Neither new phases nor changes in the interplane spacing were observed in the XRD patterns from Ag-free and Ag-doped hydroxyapatite-ciprofloxacin composites. Thus synergistic approach may work successfully thanks to the joint action of silver and an antibiotic against pathogens.



The kinetics of silver release from Ag-doped hydroxyapatite into the pH 4 and pH 7 buffer solutions under the conditions of daily analytical sampling and adding a fresh portion of buffer solution at 37 °C.

N°P3a-2

CYTOTOXIC EFFECTS OF POLYETHYLCYANOACRYLATE/CHITOSAN NANOPARTICLES

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Actually the preparation of nanoparticles intended as drug carriers is taking an important role in medical applications on cancer therapy, Parkinson disease and genetic therapies among other potential uses. They have shown some advantages over traditional therapies as tablets, suspensions and solutions. The literature shows that complex and unexpected interactions could occur between nanostructures and biological systems *in vitro* and *in vivo* and one critical issue is to control the nanoparticle cytotoxicity. The aim of this work was to study the cytotoxicity of hybrid nanoparticle systems with the combination of chitosan (Q) and Poly ethyl cyanoacrylate (PECA) on human lymphocytes and on Hela and Vero cell lines. We use MTT method for measuring cell viability and spectrophotometric measurement of malondialdehyde (MDA) by TBARS assay (thiobarbituric acid-reactive species) in order to estimate lipid peroxidation in cell line cultures. Traditional methods to evaluate the mitotic index and cell cycle progression in lymphocytes were conducted.

In this work we report the physicochemical characterization of the obtained systems and the *in vitro* cytotoxicity capacity of each system tested. All the systems showed no significant differences regarding cellular viability evaluated through MTT method neither when we estimated lipid peroxidation. No significant differences were found in mitotic index of human lymphocytes however decreased cell cycle progression was observed in the high nanoparticles dose tested.

This work showed promising potential results in the study of Poly ethyl cyanoacrylate/chitosan hybrid nanoparticle systems as drug carriers.

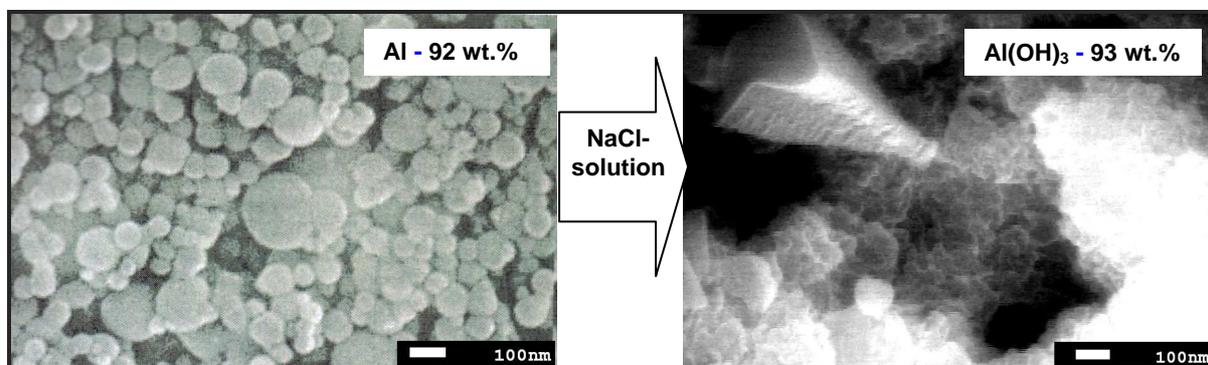
N°P3a-3

REACTIVITY OF NANOALUMINUM IN PHYSIOLOGICAL SOLUTIONS

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The production of aluminum nanopowders is constantly growing and increasing the sources of nanoparticles release into the environment. At the same time data on the high respiratory toxicity of nano-aluminum and its compounds have been accumulated in science. However, universal respiratory protective equipment for different nanoparticles has not yet developed. The development of protective equipment and regulatory documents are blocked with the lack of data on the physicochemical behavior of nano-aluminum in the respiratory tract. Electroexplosive aluminum nanoparticles have a high reactivity and it affects their basic properties (melting temperature, solubility, etc.). Therefore, it may be assumed that nano-aluminum reactivity in aggressive biological fluids, like alveolar fluid, may be extremely high.

This study was aimed at the estimation of the electroexplosive aluminum nanopowder (Al NP) reactivity in synthetic physiological solutions. Reactivity was assessed as the oxidation degree of Al NP in suspensions (0.01% wt.) based on aqueous solutions of alveolar fluid's components, such as NaCl, Na₂HPO₄, KH₂PO₄, Na₂SO₄, C₆H₁₂O₆, C₂H₅NO₂, Na₃C₆H₅O₇. It was revealed that oxidation degree of Al NP with the Al content about 90-93 wt.% reached high values in some suspensions, particularly, in NaCl-suspensions oxidation degree was 94 wt.%. According to X-ray data, the dried oxidation products contained the phase of gibbsite (Al(OH)₃). These results were related to the fact that protons (H⁺) participating in the aluminum oxidation with the formation of amphoteric Al(OH)₃, diffused through oxide layer covering bulk aluminum. However, the observation of acid-base balance in the suspensions revealed that the Al NP oxidation took place in acid medium at pH = 5.9...6.2 as well as in basic medium at pH = 7.2....7.4. Being in acid solution, Al(OH)₃ manifested basic properties; that resulted to the dissolution of produced precipitate with the formation of aluminates. These observations indicates that aluminum nanoparticles are able to dissolve in individual components of alveolar liquid, meanwhile the dissolution process may flow though the stage of the formation of insoluble precipitation.



Change of morphology (SEM) and phase composition (X-analysis) of Al NP in NaCl-suspension

N°P3a-4

DETERMINATION OF SPIONS NANOPARTICLES BIOSAFETY: PROBLEMS OF INTERFERENCES WITH CYTOTOXIC ASSAYS

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Superparamagnetic nanoparticles (SPION) have interesting magnetic properties for biomedical applications. However, these applications should exclude any risk to the human health and the environment. It was reported in several studies that the unique properties of nanoparticles (NP) could lead to unpredictable interactions with the components of nanotoxicity assays. The consequence of this interference is a false conclusion on the biosafety of NP.

Objective: To determine the possibility, and depending, the interference mechanism of three SPION (different chemical composition) with three nanotoxicity assays.

Methods: SPION were incubated in culture medium without cells for the study of the potential and the mechanism of interference with nanotoxicity assays (LDH, MTS and Presto blue). The determination of SPION nanotoxicity was performed in A549 cells using the same assays.

Results: Our results showed that SPION interacted with MTS and LDH assays. This interference was a consequence of catalytic activity of these NP which occurred by a non-enzymatic conversion of tetrazolium. Moreover, these two assays are not valid for nanotoxicity assessment of these SPION, despite the interference correction. However, no interference of Presto blue with these SPION was detected, thus validating our data showing a cytotoxicity of these NP.

Conclusion: We showed that SPION interfere with the conventional nanotoxicity assays, wondering about the data of previously published articles (no determination of interference). So, determination of potential interference is an important step that should be included in the biosafety studies of NP.

Keywords: SPION, Nanotoxicity assays, interference, A549 cells.

Supported by the Reseau en Santé Respiratoire du Québec (RSRQ)

N°P3a-5

RENAL METALLOTHIONEIN EXPRESSION BY NANO ZINC PARTICLES IN CADMIUM-TREATED RATS

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In the present study, the potential ability of different sizes (15, 20 and 30 nm) and doses of nano zinc particles (3, 10, 100 mg/kg bw), administered solely and simultaneously with cadmium chloride (2-5 mg/kg bw), to induce the synthesis of metallothionein I and metallothionein II in a rat model of the kidney was investigated. The expression of induced MT mRNA after oral exposure to nano Zn and Cd was determined by the reverse transcription polymerase chain reaction (RT-PCR). The mRNA expressions were markedly upregulated by nano-Zn 20 nm (10, 100 mg/kg bw), dose dependently. In simultaneously CdCl₂ and nano-Zn 15 nm treated rats, MT-I and MT-II RT PCR demonstrated clear bands in 100 mg/kg bw and however, undetectable to very faint bands in 3 and 10 mg/kg bw treated rats. Results of the Northern blot analysis suggested that the mRNAs of both MT-I and MT-II were induced in the kidney, in response to the simultaneous Cd and various doses of nano -Zn 20 nm or nano-Zn 30 nm exposure. Rats exposed to combination of Cd and nano-Zn 20 nm at low dose of 3 mg/kg bw induced formation of renal MT-I and MT-II. However, MT-I and MT-II mRNA were not observed in control, and CdCl₂ only treated rats. Higher mRNA expressions of MT-I and MT-II in rats supplemented with nano sized Zn particles lead us to conclude that the larger sizes and intensive doses of these nano particles can upregulate MT-I and MT-II mRNA expression in the rat kidney. Co-administration of Cd with nano sized Zn particles improves the induction of metallothionein synthesis in treated animals.

N°P3a-6

DIFFERENCES IN NANOTOXICITY RESPONSES BETWEEN HUMAN BRONCHIAL SMOOTH MUSCLE CELLS (HBSMC) OF NORMAL AND ASTHMATIC SUBJECTS FOLLOWING EXPOSURE TO QUANTUM DOTS (QDS) NANOPARTICLES

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Introduction: Quantum dots (QD) are spherical nanocrystals of 1 to 10 nm in diameter that exhibit interesting fluorescence properties for biomedical applications (e.g. optical imaging or nanovectors for drugs delivery). However, the risk of QDs on human health is poorly known, and mostly unknown in pathological conditions.

Objective: To determine the potential nanotoxicity of QDs on normal and asthmatic HBSMC.

Methodology: Normal or asthmatic HBSMC were exposed to 10 to 1000µg/ml of QD CdTe/CdS during 16 to 72h. Nanotoxicity was determined using the MTS and Presto blue assays to assess mitochondrial and cellular metabolism, respectively. The reduced and oxidized forms of glutathione were measured as markers of oxidative stress responses, and the expression of cytokines and metalloproteinases (MMP) as markers of airway inflammatory and remodelling responses, respectively.

Results: There is no interference between QDs and the cytotoxicity assays used which validates nanotoxicity data. We found that the incubation of normal and asthmatic HBSMC with QDs decreased cell viability in a concentration and time dependent manner. When cells were exposed to 1000µg/ml QDs, the decrease in cell viability at 24h post-exposure was 75% and 45% for normal and asthmatic HBSMC, respectively, in comparison to unexposed cells. This finding indicates that HBSMC are more sensitive to QD-induced cytotoxicity than asthmatic cells. In contrast, normal HBSMC have the ability to restore the oxidative balance when QDs induce oxidative stress responses, but not asthmatic cells. Interestingly, the release of eotaxin and GM-CSF was higher in asthmatic HBSMC exposed to QDs than in normal cells. Finally, the profile of MMP-1, -2 and -3 expression between normal and asthmatic HBSMC showed heterogeneity for MMPs levels and concentration-responses to QDs.

Conclusion: This study provides the first evidence on the risk of the use of QDs as nanovectors for drugs delivery on the respiratory system, and particularly in allergic airways.

Keywords: Quantum dots, Nanotoxicity, inflammation, asthma, HBSMC cells.

Supported by the Center for Asthma in the Workplace (CIHR funded) and the Réseau en Santé Respiratoire du Québec (RSRQ)

N°P3a-7

COMPARATIVE EVALUATION OF CYTOTOXICITY AND DNA DAMAGE INDUCTION BY CARBON NANOTUBES, CERIUM DIOXIDE, TITANIUM DIOXIDE AND SILVER NANOPARTICLES IN MAMMALIAN CELLS

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Keywords: Nanoparticles, cytotoxicity, genotoxicity

Nanoparticles (NPs) can be defined as structures sized in the nanoscale dimension (10^{-9} m), and its remarkable properties make them attractive to include in new products. However, few studies investigated its cyto/genotoxic potential. So there is a need to know its hazard effects before NPs incorporation into new products. In this study, we evaluated the effects of four different NPs: Silver nanoparticles (AgNP – used in products because its antibacterial effects), Carbon nanotubes (CNT – a promising material to energy field), Cerium dioxide (CeO₂NP – used as a fuel additive) and Titanium dioxide (TiO₂NP – used in sunscreen). The size of NPs was determined 0 and 24h after dispersion in culture medium using the Zetasizer Nano Z (Malvern). The AgNPs showed the largest gain in size after 24h in culture medium, followed by CeO₂NP and TiO₂NP. The opposite was observed to CNT, after 24h a decreased in size was detected. The cell viability (XTT) and colony-forming ability of four mammalian cell lines (V79, MRC5, A549 and HeLa) were investigated after treatment with these NPs. For the cell viability the cell lines were treated by 24h in the concentrations 0.1, 1, 10 and 100 µg/mL. The results detected that only CNT (10 and 100 µg/mL) induced a significant ($p < 0,05$) reduction of cell viability in HeLa and A549 cells, while to MRC5 cells a significant reduction were induced by AgNP and CeO₂NP at 100 µg/mL and by CNT in the two higher concentrations (10 and 100 µg/mL), finally to V79 cells all the NPs induced significant reduction at 100 µg/mL. In clonogenic assay a long period of NPs incubation (7 days to V79 cells and 12 days to MRC5, A549 and HeLa cell lines) in three concentrations (1, 10 and 100 µg/mL) was realized, and we observed that the colonies number reduced in all cell lines treated with AgNP at 100 µg/mL, and CNT reduced the colony-forming ability of V79 cell lines in that concentration. The DNA damage induction was evaluated by Comet assay (MRC5 and A549) after treatment with AgNP, CNT, TiO₂NP and CeO₂NP by 24h in the following concentrations 0.1, 1, 10 µg/mL. The tail intensity (% DNA in tail – Comet Assay IV[®] Perceptive Instruments) increased ($p < 0,05$) in the treatment with AgNP, CNT and CeO₂NP in the MRC5 cell line, while in the A549 cell line only oxidative damage (enzymatic detection by hOGG1) increased with AgNP and CNT. We can point out as general conclusions that NPs are a new class of toxic agents with genotoxic activities. The different NPs have different effects of cytotoxicity, more specifically AgNP and CNT are more toxic than TiO₂NP and CeO₂NP to V79, MRC5, A549 and HeLa cells. Cancer cell lines (A549, HeLa) are more resistant/tolerant to NPs than normal transformed cell lines (V79, MRC5). DNA damage induction is mainly associated to oxidative lesions as observed to A549 cell lines treated with AgNP and CNT, showing an indirect genotoxicity to NPs.

Financial Support: FAPESP Processo nº 2010/10863-8, Edital Universal-CNPq, CAPES.

N°P3a-8

GENOTOXICITY IN RTG-2 FISH CELL LINE UPON EXPOSURE TO DIFFERENT DIMENSIONS OF SILVER NANOPARTICLES DETECTED BY THE COMET ASSAY

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The increased use of nano-sized metallic materials is likely to result in the release of these particles into the environment. It is, however, unclear if these materials are harmful to aquatic animals. Furthermore, because the dissolution of such nanomaterials will occur, it is probable that some of the adverse effects resulting will result from the dissolved metal species. Genotoxicity is often one of the earliest signs of toxicant impact and is frequently assessed using in vitro models in biomonitoring programmes. In this study Comet assay and Micronucleus Test were applied to assess DNA damage in rainbow trout gonad-2 cell line (RTG-2) after 4 and 24 h of exposure to a concentration range of Silver nano particles. Cytotoxicity was tested using Trypan Blue method and MTT assay. Cell lines have been treated with 5–10–20–40–80 µg/ml of different silver-based nano compounds. The same doses of exposure were tested by Micronucleus test. H₂O₂ was used as positive control in Comet assay. Comet assay results showed that the particle superficial morphology and size had an effect on DNA integrity, as mesoporous ones induced higher damage level with respect to the dense nanoparticles. An increase in micronucleated cells was observed at intermediate doses of nano-Ag. Cell suspensions exposed to the lowest dimension of nano-Ag show a particular distribution of these particles inside the cell, with respect to the one across the slide. A deeper analysis of this phenomenon is in progress (TEM observation).

N°P3a-9

TWO “FACES” OF CARBON NANOTUBES

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The interest in carbon nanotubes (CNTs) is mainly associated with their unique properties, which make them attractive options in various medical and industrial applications. Opinions on the biocompatibility/toxicity of CNTs *in vitro* and *in vivo* environments are ambiguous. Some researchers have compared them to the negative effects of asbestos fibres, especially dangerous for respiratory tract and skin. Other researchers in turn have indicated that nanotubes are biocompatible and have a positive impact on cell growth and proliferation and due to their mobility potential in living systems; they may be successfully used as novel drug delivery systems. Analysis of the available literature shows that CNTs possess two different faces, both positive and negative in contact with a living organism and suggests that both of them are right. The differences in the interaction of CNTs with the living organism are mainly due to their synthesis process and a number of various factors which could indirectly affect cellular or tissue response. Mostly to these factors include; their lengths, functionality, their concentration, duration in the living body, catalyst impurity and agglomeration.

The aim of this study is to compare the effects of different types of nanotubes on cellular and tissue response. Four types of as prepared CNTs differ in structure, diameter, length and purity were investigated in contact with the murine macrophages (RAW 264,7) and human osteoblast (NHOst) and were implanted into the gluteal muscles of adult Wistar rats. During this investigation was also observed how the same CNTs but before and after chemical functionalization have dramatically different impact on the cells response both *in vitro* and *in vivo* conditions. Carbon nanotubes oxidized in mixture of acids are more dispersed in the culture media due to carboxyl and hydroxyl groups on their surface and simultaneously easier transferred inside the cells by the phagocytosis process and free passage by cell membrane in comparison to as prepared CNTs. Non functionalized CNTs have tendency to agglomerates which distinctly decreases cell viability as a result of frustrated phagocytosis. Cells growth and viability in contact with the same CNTs but before and after functionalization are different which confirms that their toxicity should not be generalized but any kind of CNTs should be considered separately. Some types of CNTs even improve biocompatibility of traditional materials such as polytetrafluoroethylene (PTFE) which has been observed in bone cell cultures. In contact with CNTs deposited on PTFE membrane the cells proliferated much faster and were much more flattened in comparison with PTFE membrane without modification.

Histological and histochemical reactions were carried out on muscle tissue slides containing the implanted material. We observed that hydrophilic CNTs formed small aggregates uniformly dispersed in the tissue and were well phagocytosed, while hydrophobic CNTs cumulated into large size aggregates. These aggregates were accompanied by abundant foreign body giant cells (FBGC). Hydrophilic nanotubes reduced their agglomeration in muscle and decrease of the acid phosphatase (AP) activity (and inflammation) in surrounding tissues – especially the FBGC response. The process of tissue regeneration was intense and numerous regenerating muscle fibres occurred around and between the implanted nanotubes. Fast regeneration of the muscle was observed in the vicinity of the implanted materials.

This study has been supported by the Polish Ministry of Science and Higher Education, project no NN 507402039

N°P3a-10

STUDY OF BIOCOMPATIBLE AND HEMOCOMPATIBLE PROPERTIES OF AMORPHOUS HYDROGENATED CARBON COATINGS PRODUCED BY PULSED MAGNETRON DISCHARGE.

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In this work, we evaluate the potential biomedical application of amorphous hydrogenated carbon (a-C:H) thin films deposited by reactive pulsed magnetron sputtering. Our results show a good hemocompatibility and biocompatibility: no effect on hemolysis and hemostasis were observed. Moreover, the proliferation of various cell types such as endothelial, fibroblast and osteoblast-like cells was not affected by the coating. Cell growth on a-C:H coatings is proposed to take place by a two-step process: the initial cell contact is affected by the smooth topography, whereas the polymeric-like structure, a moderate hydrophilicity and a high hydrogen content direct the posterior cell spreading, while preserving the hemocompatible behavior.

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N°P3a-11

EFFECT OF NANO-FILLER PARTICLES AND METHACRYLATE MONOMERS, SEPARATE AND IN COMBINATION, ON THE SECRETION OF CYTOKINES

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Dental composite materials are made up by filler particles (inorganic component), a resin matrix (organic component) and a coupling agent for binding the filler to the matrix. The organic component consists of monomers that polymerize upon activation by visible light illumination. Triethylenglycol-dimethacrylate (TEGDMA) and bisphenol A-glycidyl dimethacrylate (Bis-GMA) are commonly used monomers. Dental composite materials are often distinguished by the size of the filler particles. Nanocomposites are composites where part of or all particles are in the nano range (<0,1µM). Often used are silica- and zirconium nanoparticles of various sizes. Nanoparticles are used in dental composite materials to enhance its mechanical and esthetic properties.

The polymerization process is never complete, and leakage of unreacted methacrylate monomers occurs. As a result of wear and breakdown of the bond between the filler and the matrix, the filler particles can also leach from the composite. Release of unpolymerized monomers, low polymerized polymer molecules, filler particles and also alkali ions can lead to dissolution of the filler network and further release components of the material. Altogether, this can result in exposure to the oral environment to inorganic ions, unpolymerized monomers and filler particle.

We aim to investigated the effect of TEGDMA, Bis-GMA and nano-silica filler particles with mean size of 12 nm on the secretion of IL-6, IL-8 and IL1-β in BEAS 2B and monocyt-macrophage (THP-1) cell line. Measurements of Cytokines were done by Enzyme-linked immunosorbent assay (ELISA). We exposed the cells to monomers and nanoparticles alone and in combination to investigate possible additive and synergistic effects.

Preliminary result shows that nanoparticles induced a dose dependent release of IL-6 in BEAS2B cells. Bis-GMA and TEGDMA did not influence the IL-6 response alone. However, co-exposure of SiO₂ particles and Bis-GMA seems to diminish the effect compared to the particles alone while co-exposure to TEGDMA and particles seems to enhance the IL-6 response compared to the particles alone.

N°P3a-12

IN VIVO GENOTOXICITY OF TITANIUM DIOXIDE NANOPARTICLE

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Titanium dioxide (TiO₂) nanoparticles are manufactured products widely used in cosmetics but also in the food sector as additive. TiO₂ nanoparticles health adverse effects have been mostly studied on lungs. Nanoparticles exposure has been associated with a variety of lung disorders in rodents, including inflammation, fibrosis, oxidative stress, genotoxicity and also tumors. However, effects from nanoparticles ingestion are still poorly studied although TiO₂ nanoparticles seem to have negative health impacts, including genotoxicity in mice (Sycheva et al, 2011; Trouiller et al, 2009). Therefore, it seems necessary to study whether TiO₂ nanoparticles are also genotoxic in another animal model. Thus, this study proposes to expose rats during several days, to P25 TiO₂ nanoparticles (largely used in industry) and test if they could induce genotoxicity. Three groups of Sprague-Dawley rats were exposed during five days, ad libitum water mixed with TiO₂ nanoparticles at three concentrations (0,5 mg/ml, 0,05 mg/ml or 0,025 mg/ml). Rats were sacrificed 24h after the last day of exposure. The adverse effects on DNA were tested on whole blood with three assays (the Alkaline Comet Assay, the Micronuclei assay and the γ -H2AX immunostaining assay). Peripheral blood samples were taken at first before exposure, then after the five days of exposure and finally 24 h after the last day of exposure. The total dose after five days oral exposure was estimated at 12.5, 25, and 250 mg/kg. There was a significant dose-dependent increase of micronuclei in polychromatic erythrocytes. Also percentage of DNA in comets' tail was significantly increased at the highest concentration (250mg/kg) just after exposure. Finally, preliminary results from γ -H2AX immunostaining assay show a significant increase of double breaks in a dose-dependent manner. Thus oral exposure to TiO₂ nanoparticles induces different genotoxic alterations in rat's blood, from clastogenic effects to DNA single and double strands breaks. DNA breaks repair might be impossible or imperfect, thus leading to apoptosis or mutations, which could participate to cancer initiation process.

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N°P3a-13

INSIGHTS INTO TITANIUM DIOXIDE NANOPARTICLE AND FINE GENOTOXICITY IN HUMAN LYMPHOCYTES

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In this study, an evaluation of genotoxicity of anatase Titanium Dioxide (TiO₂) nanoparticle (NP) and fine (F) forms is carried out in isolated human lymphocytes using different approaches such as cytokinesis blocked micronucleus (CBMN) test that detects chromosome damage, comet assay, that highlights single (ssb) and double (dsb) DNA strands breaks and levels of 8-OxoG on DNA. The characterization of NP and F TiO₂ have been carried out by transmission electron microscopy (TEM) to determine primary size and shape and by scanning electron microscopy (SEM) for size distribution and agglomeration in lymphocyte culture medium. Moreover, the interaction between particles and human lymphocytes have been investigated using flow cytometry.

Results from TEM characterization showed primary size of 20-60nm and 70-6000 nm for TiO₂ NP and F respectively. Results from SEM, displayed comparable size distributions for the two forms, with an average diameter of 222 ± 88 nm for NPs and 658 ± 538 nm for F particles.

Interaction of particles with lymphocytes was evaluated by the side scatter (SS) parameter in flow cytometry. Preliminary results indicate the presence of a cell subpopulation (about 17% of all lymphocytes) with a dose dependent increase in SS respect to control after treatment with both NP and F forms.

For CBMN assay lymphocytes were treated for 48 hour (24 hours before cytochalasin B addition) or 28 hours (just before cyt B addition) with 50-200 µg/ml of TiO₂. No clear micronucleus induction with TiO₂ NP and a slight increase with the F was observe. Moreover no decrease in cell proliferation was also observed.

The analysis of DNA ssb and dsb by comet assay showed a significant increase (p<0.005, t-Student) in single and double strand breaks, both as tail moment and tail length values, in cells treated for 24h with 50-400 µg/ml of TiO₂ NP, more evident at lower doses. Preliminary results of treatments with TiO₂ F suggest a slight but not significant increase of both DNA damage parameters.

Preliminary results on detection of 8-OxoG level on DNA of lymphocytes treated for 6 and 24 hours with 10-100 µg/ml of TiO₂ NP highlight an increase of 8-OxoG levels in a dose dependent manner. Similar experiments with TiO₂ F are ongoing.

Further studies are on going to confirm results and to investigate the identity of the lymphocyte (or white blood cells) subpopulation that interacts with TiO₂ and the nature of DNA damage highlighted by comet assay.

N°P3a-14

HIGHLY CONCENTRATED SILICA NANOPARTICLES AFFECT THE ACTIVITIES OF NEURAL STEM CELL LINE

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Several in vivo studies suggest that highly concentrated nanoparticles (>100 nm) have ability to reach the brain when inhaled, intravenously/intraperitoneally administered, or applied on the skin. Moreover, Yamashita et al. reported the 70-nm silica particles reached to the brain of murine fetuses when the particles were intravenously administered to pregnant mice (Yamashita *et al.*, Nature Nanotechnology, 2011).

However, it is unknown whether penetrated silica particles affect neurogenesis and neuronal activity. In the present study, we observed a human neural stem cell line, as a neural progenitor cell model, cultured with silica particles (sizes: 30 nm, 70 nm, and 325 mesh microparticles (approximately < 45 μ m); concentrations: 1000, 100, and 10 μ g/mL) in 7 days. Further, we measured mitochondrial activity to investigate the metabolic influence by the particles on the neural stem cells. In addition, we investigated the expression of cytokines and brain cell markers 24 hours after the culture with 30-nm silica nanoparticles to determine the influence of contact with nanoparticles on cellular activities.

In our observation, the aggregation of the neural stem cells considered to be due to cellular injury was seen in the concentration of 100 and 1000- μ g/mL particles in all sizes. On the other hand, the mitochondrial activity showed concentration-dependent reduction and temporary elevation in cases where 30 nm and 70 nm particles were used, respectively. However, little influence on the metabolic activity was observed in case of the micrometer particles. Moreover, investigation of comprehensive cytokine expression 24 hours after culture with the 30-nm particles resulted in the expression of some cytokines related to neural differentiation.

These results indicated that silica nanoparticles may influence the proliferation of neural stem cells and the expression of cytokines after penetrating the brain.

This study was supported by Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan (H22-chemical-young-009).

N°P3a-15

**TOXICOLOGICAL INFLUENCE OF GIVING THE SILICA NANOPARTICLES ON CULTURED
CENTRAL NERVES CELLS**

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Nanotechnology is developing rapidly in the world and the production of novel man-made nanoparticles is increasing. It is possible that various nanoparticles by the peculiarity of the particle size and by the surface property have the potential to affect risky our human health. The silica nanoparticles reach the brain in vivo, thus it is important to establish evaluating methods for the influence of the nanoparticle on the central nerves.

We investigated the toxicological influence of touching nanoparticles directly to the cortex neurons from embryonic wistar rats at 18 days of gestation. Cortex neurons DIV (Days in vitro) 21 were treated with 30 nm-silica conjugated Rhodamine or non-labeled 12 nm-silica for 1h. We used three concentrations (0.01mg/ml, 0.1 mg/ml and 1.0mg/ml). These cultured cells were dipped into the media containing the stimulants (or no stimulants for the controls) for 1h. Dendrites of the neurons were dyed by anti-MAP2, which specifically dye the microtubule-associated protein2. These neurons were also dyed with anti-Rhodamine-caspase3/7. Caspases are a family of cysteine proteases that act in concert in a cascade triggered by apoptosis signaling. The culmination of this cascade is the cleavage of a number of proteins in the cell, followed by cell disassembling, cell death, and removal of the cell debris by phagocytes ultimately. Many neurons were damaged especially in dendrites, and died immediately after treating 0.01 mg/ml silica nanoparticles. It needs to determinate the safe concentration of the silica nanoparticles to the central nerve cells.

This study was supported by Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare of Japan (H22-chemical-young-009).

N°P3a-16

EFFECT OF SURFACE MODIFICATION ON ZN IONS AND ROS PRODUCTION OF ZNO NANOPARTICLES

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In recent years, there has been a huge increase in the use of ZnO nanoparticles (NPs) in various commercial products, such as sensors, solar cells, transparent electrodes, electroluminescent devices, and sunscreens. ZnO can absorb both UV-A and UV-B radiation and therefore offers a better protection than TiO₂. The estimated production rate of metal oxide (TiO₂, ZnO, Fe₂O₃) nanoparticles for skincare products is 10³ tonnes per annum.¹ There are even more nanoparticles used in structural, biotechnology, instruments, environmental and other applications. It is expected that in future, more and more nanoparticles will be used in power, engineering, and electronic industries.¹ A report suggested that at least 25% of the amount of the sunscreens applied onto the skin are washed off,² which implies that more than 250 tonnes of nanoparticles from sunscreens are probably released into the environment each year.

Though ZnO nanoparticles products have been on the market for many years, the cytotoxicity of the nanoparticles is not fully understood. In some reports, it is proposed that the antimicrobial properties are the result of release of metal ions.³ Other studies also suggested the reactive oxygen species (ROS) as the mechanism responsible for antimicrobial properties.⁴ However, most toxicity studies are performed on bare nanoparticles. Since in practical applications nanoparticles usually have modified surface, we have studied the effect of surface modification on Zn ion release and ROS production. Since these properties are expected to significantly affect their toxicity. Following materials have been used for surface modification: dodecyl-trichlorosilane, 3-methylthiophene, 1,8-octanedithiol and 1-dodecanethiol. ROS production is detected using electron spin resonance (ESR) and spin traps (DMPO and DEPMPO), while metal release is obtained by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results are discussed in detail.

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N°P3a-17

CYTOTOXICITY AND GENOTOXICITY OF SILVER NANOPARTICLES WITH DIFFERENT SIZES IN MAMMALIAN CELLS

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key-words: silver nanoparticles, clonogenic assay, genotoxicity

The special chemical, thermal, electrical and optical characteristics of nanoparticles (10^{-9} m) are being used in a range of products but it is known that these particles can cause adverse biological effects, which has led to concerns about risks to health and to the environment resulting from the use of these particles. Recently, silver nanoparticles (AgNPs) has been the target of cytotoxicity and genotoxicity studies due to its wide use in the pharmaceutical industry and in the manufacture of many products. So, this study evaluated the cytotoxicity and genotoxicity of AgNPs of 10 and 100 nm in the cell lines CHO-K1 and CHO-XRS5. Both strains are Chinese Hamster fibroblasts, the difference between them is that the CHO-XRS5 has a mutation in the gene *xrcc5* and due to this mutation this cell line cannot repair double strand breaks. So, we hypothesize that double strand breaks induced by AgNPs could affect mainly CHO-XRS5 when compared to CHO-K1. The zeta potential and size of AgNPs was determined using the Zetasizer Nano Z (Malvern). The cells cultures were exposed for 24 hours to different concentrations of AgNPs ranging from 0.025 to 5 μ g/ml. Cytotoxicity was evaluated by XTT and clonogenic assay and genotoxicity was detected by Comet assay with and without enzyme hOGG1 to also detect oxidative damage. The results of Comet assay were analyzed using the software Comet assay IV (Perceptive Instruments Ltd.) and % DNA in tail - tail intensity was used as DNA damage parameter of the treatments. The AgNPs of 100nm presented low values of the polydispersity index (PDI) and high negative charges compared with the AgNPs of 10nm. The CHO-XRS5 cells show a lower colony forming ability (clonogenic assay) compared with CHO-K1 cells after treatment with AgNPs and in both cell lines it was observed a decrease of cell colony-forming ability with the increase of concentrations. We observed a decrease of more than 67% of cell viability (XTT) in both CHO-K1 and CHO-XRS5 cells treated with AgNPs of 10 and 100 nm in the concentration 5 μ g/ml. The preliminary results with CHO-K1 cell line show low levels of DNA damage in the control cultures [9.48% and 12.87%(hOGG1- oxidative damage)] and extensive DNA damage at concentrations 2.5 μ g/ml (35.52% and 27.04%(hOGG-oxidative damage)) and 5 μ g/ml (36.83% and 35.7 % (hOGG1-oxidative damage)). A dose response was clearly seen in both assays. The preliminary results show us a strong effect of cytotoxicity and genotoxicity of AgNPs with two different sizes in CHO-K1 cells and CHO-XRS5, proving that the short-term effects of these particles can cause DNA damage and its long-term effects can cause cells death, representing concerns to health and to the environment.

Financial Support: FAPESP n° 2010/10863-8, Edital Universal - CNPq, Capes

N°P3a-18

**IN VIVO TOXICITY OF ENOXAPARIN ENCAPSULATED IN MUCOADHESIVE NANOPARTICLES:
TOPICAL APPLICATION IN A WOUND HEALING MODEL**

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Wound healing is a dynamic and complex process that results in functional and anatomical restoration of the injured area. It comprises four phases including hemostasis, inflammation, proliferation and tissue remodeling. There are some pathological conditions that impair healing, such as diabetes. Fifteen percent of diabetic patients have topical ulcers that are very difficult to treat. An interesting drug for treatment of these ulcers is low molecular weight heparin (enoxaparin) due to its anti-inflammatory property, analgesia, epithelization, and wound healing effects. The encapsulation of drugs, such as, enoxaparin, in nanoparticles can help in wound healing, increase drug stability, decrease drug toxicity and increase drug permeation and adhesion in the skin. The aim of this work is to evaluate the toxic effects of enoxaparin (ENOXA) encapsulated in mucoadhesive nanoparticles in an *in vivo* wound healing model in normal and diabetic rats. Nanoparticles were prepared by double emulsion method. The size, surface charge and stability of these particles were analyzed. Formulations of natrosol gel with or without nanoparticles were prepared for *in vivo* tests. In this assay, an excisional wound (1 cm²) was made with scissors, after shaving the animal dorsum under anesthesia. The animals were divided in different groups: G1-gel free ENOXA, G2-Sham, G3-pure gel, G4-empty nanoparticles gel and G5-ENOXA nanoparticles gel. The animals were treated once by a day until the day of sacrifice (10 days after the lesion). The blood and skin of animals were removed for toxicity analysis. Oxidative stress in animal tissues (nitrite and TBARS) was analyzed and the renal and hepatic toxicity was evaluated through analysis of creatinine and urea and transaminases, respectively. Furthermore, hematological parameters and dosage of anti-Xa to demonstrate the presence of ENOXA in blood, was done. Our results showed a higher concentration of NO₂ and TBARS in tissue of animals treated with ENOXA. All values of creatine (0.46±0.03), urea (51.54±3.64), ALT (45.54±3.50) and AST (135.95±15.29) were in the reference range indicating that formulations did not cause renal or hepatic toxicity. Furthermore, there were no significant differences in urea, creatinine and aminotransferases levels and in the hematological parameters between the control and all treated groups. The animals treated with mucoadhesive nanoparticles with ENOXA showed a minor wound (0.034 cm²) compared with the group treated with free ENOXA (0.091 cm²), being statistically significant (p=0.0238) according the Mann-Whitney test. In 14 days the wound was completely healed. The anti-Xa test showed that ENOXA did not reach the bloodstream, demonstrating that these formulations were safe for topical application, without side effects. ENOXA administered by the intravenous pathway can cause thrombocytopenia, bleeding and hypersensitivity reactions. This study demonstrated an interesting formulation based on mucoadhesive nanoparticles with enoxaparin that improved wound healing and did not exhibit *in vivo* toxicity. Furthermore, the formulation showed a good effect on wound healing in diabetic animals, indicating a promising treatment for ulcers.

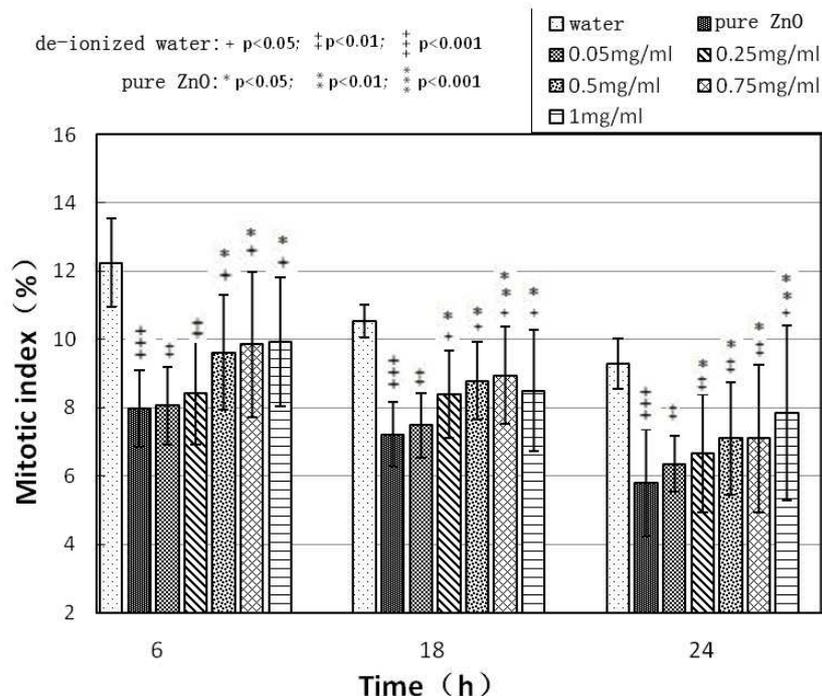
N°P3a-19

PHYTOTOXIC AND GENOTOXIC EFFECTS OF PVP COATED ZNO NANOPARTICLES ON GARLIC (ALLIUM SATIVUM L.) ROOT TIP CELLS

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Zinc oxide (ZnO) nanoparticles have been used in a large variety of commercial products such as cosmetics and sunscreens, at the same time, they have also resulted in a growing public debate on the toxicological and environmental effects of direct and indirect exposure to these materials. According to recent toxicological study, ZnO NPs has potential toxic effects in cells, plants, animals and ecologies. Although, studies on the toxicity of ZnO NPs are growing fast, ZnO NPs' toxic mechanism is still unclear, and there is no consolidated solution to its toxicity. Therefore, in order to improve the understanding about the mechanism of ZnO NPs' toxicity, we studied the effect of PVP coating on the toxic effect of ZnO NPs on the mitosis of garlic root tip cells.

3-5 nm of ZnO NPs were synthesized via sol-gel method and suspended in de-ionized water. The concentration of the ZnO NPs' suspension was selected to be two times the fifty percent inhibitory concentration (IC₅₀) and various concentrations of PVP solutions were used for the surface modification of ZnO NPs. The coated and uncoated ZnO NPs' suspensions were then used to cultivate garlic roots. After 6, 18 and 24hrs of cultivation, the effects of PVP coating on the toxicity of ZnO NPs on the root growth, root apical meristem mitosis and mitotic aberrations of garlic were analyzed. The results showed that surface modification with 0.25mg/ml or higher concentrations of PVP suppressed the toxicity of ZnO NPs on the growth of garlic root and the mitosis of root tip cells in some degree. To the mitosis of root tip cells after 18 h and 24 h of incubation, the toxicity of ZnO NPs modified with higher concentrations of PVP (0.75mg/ml and 1.0mg/ml) were suppressed remarkably. This was statistically significant at P<0.01. For the effects on the chromosome aberration rate, pure ZnO NPs were obviously negative. Although, there are some difference in the toxicity of pure ZnO NPs and surface coated ZnO NPs on the chromosome aberration rate, the difference were more complex and depended on the concentration of PVP and the cultivation time.



Effect of PVP coated ZnO nanoparticle suspensions on the mitotic index of garlic root tip cells. Vertical bars denote standard error (SE).

N°P3a-20

INTERNALISATION OF ALUMINIUM OXIDE NANOPARTICLES INTO HUMAN CELLS: IMPACT OF PARTICLE SIZE ON THE QUANTITATIVE UPTAKE

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Today the potential risks of nanoparticles on human health are of huge scientific interest. Several investigations dealing with different methods of measuring uptake pathways of nanoparticles into cells are known. However, it is important to quantify the real uptake by useful analytical standard methods. This study examined the particle uptake of three different sized aluminium oxide nanoparticles (Al_2O_3) into human cells. Single nanoparticle size ranges from 14 nm (Alu 1), 111 nm (Alu 2) up to 2,5 μm (Alu 3). The chemical and physical particle properties were completely characterized, especially regarding the behavior in suspensions and cell culture media. Human keratinocytes (HaCaT) and human lung epithelial cells (A549) were used for cell particle treatment. Exposure was performed either in standard vessels or on glass slides. First, the investigated nanoparticles were tested for possible cytotoxic effects by fluorescence cell viability tests. By fluorescence indicator dyes (AlamarBlue and CDFA,AM) and evaluation by a GENios fluorescence reader no toxicity could be observed even at the highest test concentration of 50 $\mu\text{g}/\text{ml}$.

Nanoparticle uptake was studied by flow cytometry (FACS). Depending on size and structure of particles a difference in granularity and consequently in the side scatter signal (SSC) could be detected.

To determine the aluminium amount in the cells, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) of the cells on glass slides and thin layers of spiked agarose gels as references were used. Results exhibit clearly the nanoparticle size independent internalisation into cells. However, particles of the middle size-range were indicated as the particles which are preferably taken up by both cell lines. In general, the human lung epithelial cells show higher particle uptake rates in comparison to the human keratinocytes (see Fig. 1). Additionally, these results will be checked by indirect digestion of the treated cells with chlorine under pressure and subsequent analysis by ICP-MS.

Our results show that particle uptake by cells is highest for particles ~100 nm. Perhaps, there are varying uptake mechanisms used by cells for the internalisation of different sized nanoparticles. Conceivable reasons might be the agglomeration potential of particles smaller than 100 nm, the crystal structure and surface characteristics of the nanoparticles.

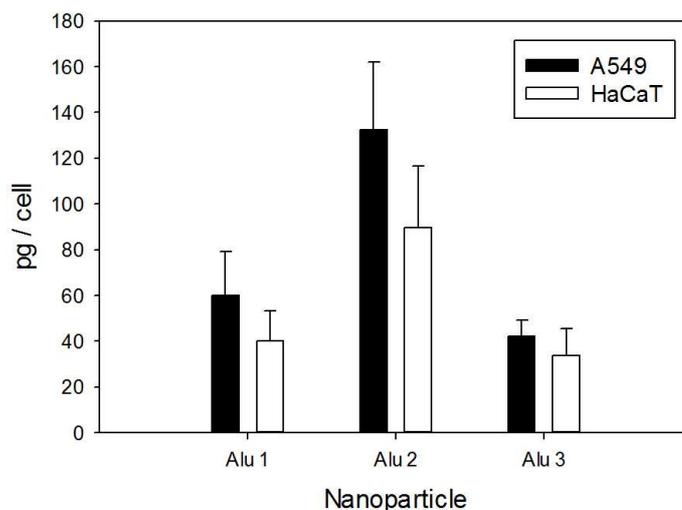


Fig. 1 : Concentration of three different sized aluminium oxide nanoparticles of human keratinocytes (HaCaT) and human lung epithelial cells (A549) on glass slides

N°P3a-21

**IMMUNOLOGICAL ASSAYS AS AN OPPORTUNITY OF ASSESSMENT OF HEALTH RISKS
OF AIRBORNE PARTICLE MIXTURE INCLUDING NANOPARTICLES**

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Nanoparticles have always been present in airborne solid matter, but contemporary development of engineered nanoparticles, together with an increasing number of unintentionally produced nanoparticles, draw attention to consequent adverse health effects. The situation is complicated by the fact that air pollution is composed of particles of varying size and composition and certain effects may be caused by “chemical cocktail effect” rather than by single material impact. That is why there is a need for a method able to show even non-specific effects of such multicomponent mixture.

The aim of this pilot study was to evaluate perspectives of the assessment of non-specific biological effects of airborne particulate matter including nanoparticles using appropriate immunological assays. Immune system reacts to changes both in homeostasis inside the body and the environment. Therefore, there is a presumption that an appropriate combination of immunological assays should present a suitable screening tool for the assessment of harmful airborne particulate matter action on the human health.

Selected assays allow us to monitor activation of various immune system components and functions. To assess comprehensive interactions and effects, the assays were performed in whole blood cultures from three healthy volunteers and we used an original airborne particle mixture from high pollution period in Ostrava region, which belongs to areas with the most polluted air in Europe. The size distribution analysis of the tested sample showed predominance of nanoscale particles in terms of particle number.

Even if certain effects were observed, the results of the immunological assays did not prove clinically significant effects of airborne particles on immune cells' functions of healthy persons. The particulate matter sample did not exhibit allergenic properties in the basophil degranulation assay, did not increase phagocytic activity in the chemiluminescence assay and did not stimulate T-lymphocyte proliferation in the lymphocyte proliferation assay. Likewise quantitative evaluation of changes in production of 25 cytokines by a multiplex technology ALBIA (addressable laser bead immunoassays) resulted in no obvious stimulation of the immune system functions.

The results of our experiments are related to short-term action of the real airborne particle mixture on the cultivation of the whole blood of healthy people. However, obtained data indeed do not exclude health risks of long-term exposure to airborne particles, especially for individuals with genetic predisposition to certain diseases or already existing disease.

This study emphasizes the in vitro assessment of complex effects of airborne particles in conditions similar to actual ones in an organism exposed to particle polluted air. Based on the experiments, we can assume that the results of immunological methods can serve as an indicator of harmful biological effects of various types of particles in the air.

N°P3a-22

**ALTERED CHARACTERISTICS OF DIFFERENTLY FUNCTIONALIZED SILICA NANOPARTICLES
IN VARIOUS ENVIRONMENTS WITH POSSIBLE IMPLICATIONS FOR BIOLOGICAL IMPACTS**

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Engineered silica nanoparticles (SiO₂ NPs) are increasingly used in diagnostics and biomedical research due to their simple production and relatively low costs. They are considered safe and produced on an industrial scale as components of a growing number of commercial products. Despite the potential benefits, there is a concern that exposure to certain types of SiO₂ NPs may lead to significant adverse health effects, thus toxicological studies on SiO₂ NPs have recently begun to increase. Nevertheless, most of those studies have used poorly characterized particles in terms of their structure and physicochemical properties. Moreover, there are little data available on their stability in media used for toxicity tests. Many NPs characteristics, especially the state of agglomeration, can be affected by the chemistry of the surroundings and the presence of both inorganic and biological moieties. It is getting known that nanoparticles inserted into biological fluids associate with biomolecules, especially proteins, organized into the 'protein corona'. All those parameters can affect an exposure dose, nature of the studied material and have a direct impact on the toxicology data.

The aim of the present work was to examine characteristics of differently functionalized 50 nm SiO₂ NPs (-NH₂, -SH and -PVP) in variety of aqueous/biological environments and assess their ability to adsorb serum proteins from culture media. For purpose of these studies, the silica NPs were carefully synthesized and deeply characterized using variety of physicochemical methods including zeta potential, DLS, TEM, SEM, AC, BET, XPS, SIMS and XRD. Interactions of the particles with biological media were additionally investigated by SDS-PAGE and extracted proteins were assessed using MALDI-TOF technique. Detected proteins were classified according to their physicochemical and biological properties.

It was recognized that all of the silica NPs, tend to agglomerate/aggregate after relatively short time of their incubation in buffers and biological media. The aggregation depended on the incubation time, NPs functionality and their concentration. The aggregation was much diminished in the medium containing serum, mainly because of the presence of proteins. The 'protein corona' formation was time and NPs functionalization depended and varied in different kind of serum.

Remarkably, surface properties were found to play a very significant role in determining the NPs behavior in different environments. Ionic strength, pH and biological macromolecules of the surroundings completely transformed the NPs surface properties and potentially the toxic effects of the nanomaterials.

N°P3a-23

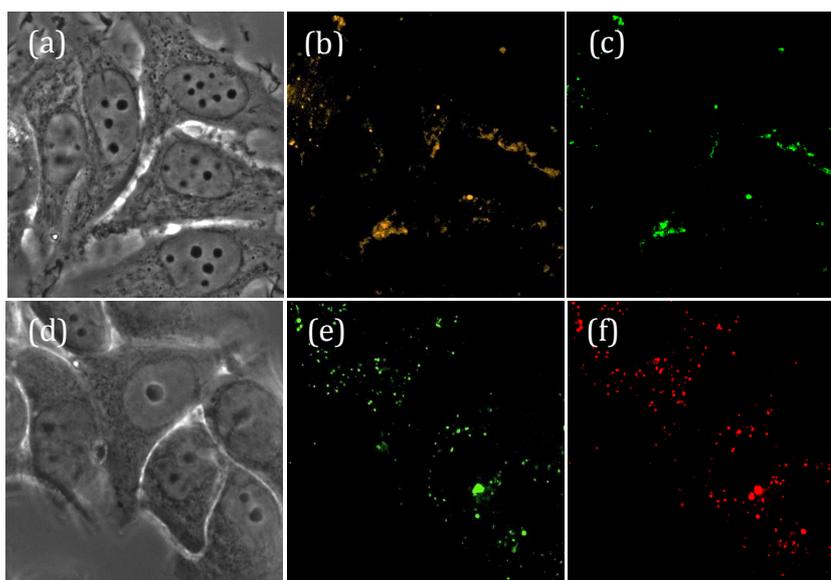
ENHANCED ANTIPROLIFERATION OF CANCER CELLS BY BIOCOMPATIBLE MULTIFUNCTIONAL MICROBIAL EXOPOLYSACCHARIDE STABILIZED MAGNETIC NANOPARTICLES

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Magnetic nanoparticles (MNPs) have emerged as one of the significant revolutionary nanomaterial for a variety of applications. The next generation MNPs with theranostic applications have attracted extensive attention. Advances in cancer nanotechnology increase the use of novel therapeutic strategies such as nanotheranostics, which utilize individualized diagnostic therapy. MNPs have been utilized as nanocarriers for drugs, contrast-imaging agents in magnetic resonance imaging, in local hyperthermia, and magnetic targeting. Also, in comparison to the traditional therapies of cancer like chemotherapy and radiotherapy, hyperthermia in general and MHT in particular can reduce severe side effects caused to normal tissue.

The effective coating of MNPs with biocompatible components adds advantage to MNPs for their enhanced circulation and application *in vivo*. Microbial exopolysaccharides (EPSs) are highly heterogeneous polymers produced by fungi and bacteria and have recently been attracting considerable attention owing to their potential applications in many fields, including biomedical research. We coated the synthesized magnetic nanoparticles with gellan gum and mauran, two bacterial EPS to enhance the biocompatibility. Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by *Pseudomonas elodea*. On the other hand, halophilic bacteria are extremophiles and the properties of their EPS seem to offer numerous applications in various fields. Mauran is an anionic, sulfated heteropolysaccharide with a high uronic-acid content, synthesized by halophilic bacterium *Halomonas Maura* in our lab.

EPS stabilized MNPs was made multifunctional by attaching targeting moiety, folate and encapsulated with a potent anticancerous drug, 5-FU. We conjugated an imaging moiety along with nanocomposite to study the effective uptake of nanoparticles. The cytocompatibility of EPS stabilized MNPs and synergistic effect of magnetic hyperthermia and drug for enhanced antiproliferation of cancer cells was also evaluated.



Entry of EPS stabilized MNPs in MCF7 cells : (a, d)-Brightfield images of MCF7, (b)-Uptake of mauran stabilized MNPs, conjugated with ciprofloxacin, (e)- Uptake of gellan gum stabilized MNPs, conjugated with FITC, (c, d) Lysosome mediated uptake of MNPs, (c) LysoTracker green, (f) LysoTracker red

N°P3a-24

**COPPER(II) OXIDE NANOPARTICLES PENETRATE INTO HEPG2 CELLS, EXERT
CYTOTOXICITY VIA OXIDATIVE STRESS AND INDUCE PROINFLAMMATORY RESPONSE**

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The potential toxic effects of two types of copper(II) oxide (CuO) nanoparticles (NPs) with different specific surface areas, different shapes (rod or spheric), different sizes as raw material and the same hydrodynamic diameter in suspension, were studied on human hepatocarcinoma HepG2 cells. Both CuO NPs were shown to be able to enter into HepG2 cells and induce cellular toxicity by generating reactive oxygen species. CuO NPs increased the abundance of several transcripts coding for pro-inflammatory interleukins and chemokines. Transcriptomic data, siRNA knockdown and DNA binding activities suggested that Nrf2, NF- κ B and AP-1 were implicated in the response of HepG2 cells to CuO NPs. CuO NPs incubation also induced activation of MAPK pathways, ERKs and JNK/SAPK playing a major role in activation of AP-1. In addition, cytotoxicity, inflammatory and antioxidative responses and activation of intracellular transduction pathways induced by rod-shaped CuO NPs were more important than spherical CuO NPs. Measurement of Cu²⁺ released in cell culture medium suggested that Cu²⁺ cations released from CuO NPs was involved only in a small extent in the toxicity induced by these NPs on HepG2 cells.

N°P3a-25

ELECTRICAL MEASUREMENT OF THE INTERACTIONS BETWEEN GOLD NANOPARTICLES AND BIOLOGICAL MEMBRANE

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Potential impact of nanotechnologies on biomedical sciences have brought an active development of various nanomaterials for diagnostics and therapeutic applications. Those nanomaterials interact effectively with the biological components such as membrane, proteins and nucleic acids. Gold nanoparticle is one of the most successfully utilized nanomaterials in cancer therapies. For this kind of bioapplication gold nanoparticles are required to pass cell membrane either by endocytosis or by direct penetration into the target cellular compartment. Therefore understanding how the nanoparticles pass through the plasma membrane of cell is a key to design the most effective therapeutic agent as well as to minimize unwanted cytotoxicity to healthy human cell and environment. There have been many techniques developed so far to monitor the interaction of gold nanoparticles with biological membrane. These techniques employing dye diffusion in liposome, scanning probe microscopy, electron paramagnetic resonance and variety of biological assays have suggested that the interaction of nanoparticles with lipid membrane induced the permeability of cell membrane as well as cell lysis at certain concentration. Here we report a new approach to monitor the change of permeability or formation of holes on lipid membrane in the presence of gold nanoparticles by measuring the current through the lipid membrane. Bilayer membrane of diphytanoyl phosphatidylcholine (DPhPC) was formed across a 0.1-mm-diameter orifice separating two buffer filled compartments. Ionic current through the lipid membrane was measured after gold nanoparticles with a diameter of 10 nm coated with charged or neutral compounds were introduced into one chamber. Cationic nanoparticles were shown to interact strongly with lipid membrane resulting in current burst through suspended lipid membrane. The interaction of nanoparticle with lipid membrane temporary disrupted the integrity of lipid bilayer and induced current leakage through the membrane. The interaction mechanisms of gold nanoparticles with lipid membrane were further investigated through analyzing electrical signals triggered by the presence of various nanoparticles. In combination with conventional methodologies this approach should find useful applications in studying detailed mechanisms of nanoparticle interaction with biological membrane and potential cytotoxicity of the nanoparticles.

N°P3a-26

METAL HOMEOSTASIS INTERFERENCES IN HEPATOCYTES CELLS TREATED BY CUO NANOPARTICLES

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The toxicity of CuO nanoparticles (NP-CuO) and their mechanism of action on hepatocytes (HepG2) were investigated in this study. In the liver, these cells play a central role in the detoxification of copper (Cu) and may be the first line of defence in case of exposure to NP. Thus, it was interesting to study the effect of NP-CuO versus CuCl₂ on HepG2 to see if there is a specific *nano*-effect.

Coated NP-CuO (1% PVP40 polyvinylpyrrolidone) have a significant toxicity compared to CuCl₂ at the same Cu concentration (10-800µM). We choose to work at 250µM Cu, a concentration which induces 20% mortality (DL20) with NP-CuO, although the Cu salt has no effect.

Electron microscopy images of sections of HepG2 cells showed NP-CuO in cell vesicles and around invaginations of the plasma membrane. This strongly suggests NP-CuO uptake by endocytosis. The expression of the genes encoding metallothioneins, heme oxygenase, glutamate cysteine ligase modifier subunit and heat shock protein 70 was analysed by qPCR. All these genes are known to be involved in Cu excess and/or oxidative stress responses. We clearly observed a higher increase in these genes expression in NP-CuO treated cells, as compared to CuCl₂. This suggests an increase in the intracellular Cu concentration due to Cu release from NP-CuO. Indeed, ICP-MS measurements of Cu released from NP-CuO showed that at pH6 there is more release than at 7.98, the culture medium pH.

Altogether our data demonstrate a putative impact of NP-CuO on Cu homeostasis and suggest a Trojan horse mechanism. Namely, NP-CuO uptake through a vesicular pathway would result in an acidic solubilisation of Cu ions and therefore interfere with Cu homeostasis.

N°P3a-27

**TRANSNATIONAL ACCESS AT VITO THROUGH THE FP7 QNANO RESEARCH
INFRASTRUCTURE**

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QNano aims to establish a pan-European analytical research infrastructure whose purpose is to drive high quality research and testing practices for assessment of the potential risks posed by nanomaterials. This four year EU funded project (Grant Agreement N° INFRA-2010-262163) started in February 2011 and comprises 27 top European analytical & experimental facilities in nanotechnology, medicine and natural sciences.

QNano's core aim is the creation of a 'neutral' scientific & technical space in which all stakeholder groups can engage, develop, and share scientific best practice in the field. Initially it will harness resources from across Europe and develop efficient, transparent and effective processes. Thereby it will enable provision of services to its users, and the broader community, all in the context of a best-practice ethos. This will encourage evidence-based dialogue to prosper between all stakeholders. However, QNano will also pro-actively seek to drive, develop and promote the highest quality research and practices via its Joint Research Activities (JRA), Networking Activities (NA) and provision of Transnational Access (TA) functions, with a global perspective and mode of implementation.

The QNano TA component is dedicated to providing Users from the European nanosafety community access to nanomaterials processing, characterisation and exposure assessment facilities. Access to 15 major European research sites is via a single application and evaluation process. The transnational access facilities at VITO for *in-situ* and *ex-situ* nanoparticle characterisation (material characterisation and imaging, mass spectrometry, and chemical analysis) and nanoparticle exposure assessment (*in vitro* assay, proteomics and transcriptomics platforms, and occupational exposure measurements and test chamber) will be highlighted. Detailed information is available at <http://www.qnano-ri.eu/access/equipment-by-taf/belgium-vito.html>.

N°P3a-28

SOURCE, ACTIVITY AND TOXICITY OF NANOMATERIALS FOR BIOLOGICAL SYSTEMS

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Since humans have always been exposed to ultrafine particles originated from sandstorms, volcanic eruptions and other natural phenomena, our biological system has become compatible with such potentially dangerous phenomena. The Reticuloendothelial system of our body, for instance, neutralizes and removes all the foreign particles including animate (such as viruses and bacteria) and inanimate objects effectively.

Furthermore, it is more than thousands of years that different types of particles such as soot and fluff originated from burnt materials and yarn and cloth respectively, have been produced by humans. Also, the recent developments in industry and public transportation which need combustion of huge amount of disparate fuels have considerably increased the production of environmental pollutions. Moreover, technological developments have changed the specifications of newly developed pollutant particles such as nano-scale materials with different chemical properties which have been produced through recent decades (except a few cases).

In this paper, health and hygienic concerns arising from new and astonishing science of nano materials are studied to increase our knowledge on the toxicity of nano materials specifically for researchers and producers.

Keywords: Nano materials, Activity, Toxicity, Biological Systems, Life cycle.

N°P3a-29

TiO₂ NANOPARTICLES AND BULK MATERIAL STIMULATE HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Nanomaterials have been increasingly produced and used within the last years and consequently the potential exposure of humans to nanoparticles has risen as well. Because of their small 1-100nm size, the physicochemical properties of nanomaterials may be different from bulk materials and may pose a threat to human health. Only little is known about the effects of nanoparticles on the human body and therefore it seems to be important to investigate possible harmful effects of nanomaterials on vital biochemical processes like the immune response.

The pro-inflammatory cytokine interferon (IFN)- γ is released in the course of cellular immune response mediated by Th1-type T-helper cells and among others induces the expression of enzymes GTP-cyclohydrolase I (GCH) and indoleamine-2,3-dioxygenase (IDO) in human macrophages and dendritic cells. GCH induces the production of neopterin, while IDO catalyses the degradation of the essential amino acid tryptophan to kynurenine. Both biochemical processes are closely related to the course of diseases like infections, atherogenesis and neurodegeneration.

In this study, we investigated the effects of TiO₂ bulk material and nanoparticles on Th1-type immune response using the in vitro model of peripheral blood mononuclear cells (PBMC). Upon stimulation of PBMC with 10 μ g/ml phytohemagglutinin (PHA), neopterin formation and tryptophan breakdown increase significantly (Jenny et al., 2011).

PBMC were exposed to rising doses of bulk TiO₂, OCTi60 (10 nm diameter) and commercial P25 (25 nm diameter) TiO₂ nanoparticles and were stimulated or not with PHA. While P25 TiO₂ nanoparticles had only little influence on neopterin formation, bulk material and OCTi60 TiO₂ nanoparticles increased neopterin production in the supernatants of unstimulated and stimulated cells significantly, the effects were stronger in OCTi60 TiO₂ nanoparticle preparations compared to bulk material. No effects of TiO₂ preparations on tryptophan breakdown were determined in unstimulated cells, in stimulated cells a slight increase was found at low concentrations for all preparations. At the highest concentrations used even an inhibitory effect on IDO activity was observed.

To conclude, OCTi60 TiO₂ nanoparticles and bulk material stimulated the formation of neopterin in PBMC, P25 TiO₂ nanoparticles had no such effect. Thus, there are distinct differences between the preparations. The parallel inhibitory influence on IDO suggests that the net effect of the tested particles would be even stronger pro-inflammatory when the immunosuppressive activity of IDO is absent. However, this study can be considered as pilot only and clearly, for a firm conclusion further experiments are needed.

N°P3a-30

IN VITRO TESTING OF NANOPARTICLES DISSOLUTION

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An increasing production of zinc nanopowders, promising materials in science and manufacturing, cause the increasing dispersion of nanoparticles into our environment, particularly into the ambient air. Through the air nanoparticles are able to enter alveolar sacs, the bottom of human lungs, in which particles are exposed to the aggressive alveolar medium. Since water-soluble nanoparticles may generate molecules and ions of new chemical composition during dissolution, the solubility of nanoparticles is one of the key factors determining nanoparticles toxicity.

In this study electroexplosive zinc nanoparticles (62 nm, 26 wt. % – ZnO, Fig.1) were exposed to the synthetic alveolar fluid to assess the dissolution degree and dissolution rate, and to determine the dissolution mechanism. The suspensions with nanopowder concentration 0.1 wt. % were prepared with the help of synthetic fluid consisting Na_2HPO_4 , MgCl_2 , Na_2SO_4 , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, NaOH , $\text{C}_6\text{H}_8\text{O}_7$, $\text{H}_2\text{NCH}_2\text{COOH}$, $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, $\text{C}_4\text{H}_4\text{O}_6\text{Na}_2 \cdot 2\text{H}_2\text{O}$, and $\text{C}_3\text{H}_3\text{O}_3\text{Na}$. Stripping voltammetry was used to determine Zn^{2+} -concentration in solutions after exposure.

It was demonstrated that maximum dissolution degree of zinc nanoparticles, namely 99.4 %, achieved in 4 hours (Fig. 2). The high value of dissolution degree meant that there was the dissolution of zinc (bulk) in the alveolar solution accompanied with the dissolution of zinc oxide (coating).

Due to the experimental data and theoretical comparison, it was concluded that the complex compounds of oxyacids with Zn^{2+} ions were most likely to form. Concerning the stability of generated complexes, it depends upon the pH level. The stability constant of tartrate complex is $2.2 \cdot 10^3$ at $\text{pH} = 3.35$ and the stability constant of citrate complex is $8.3 \cdot 10^{10}$ at $\text{pH} < 6.6$.

Alongside, the mechanism of zinc nanoparticles dissolution related to the electroexplosive zinc nanoparticles completely dissolving in the synthetic alveolar fluid has been proposed. As discussed above, the dissolution was suggested to include the following stages. The first stage comprises the formation of amphoteric zinc hydroxide in the oxygenated suspension of zinc nanoparticles and the synthetic alveolar fluid. The second stage includes the dissolution of zinc hydroxide with the formation of soluble zinc salts. At the third stage the formation of citrate and tartrate complexes of Zn^{2+} ions has been proposed.

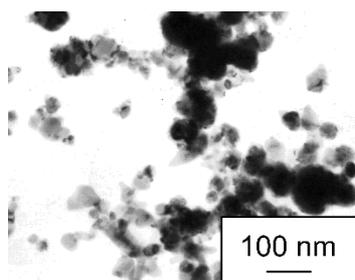


Fig 1. TEM-image of Zn nanoparticles

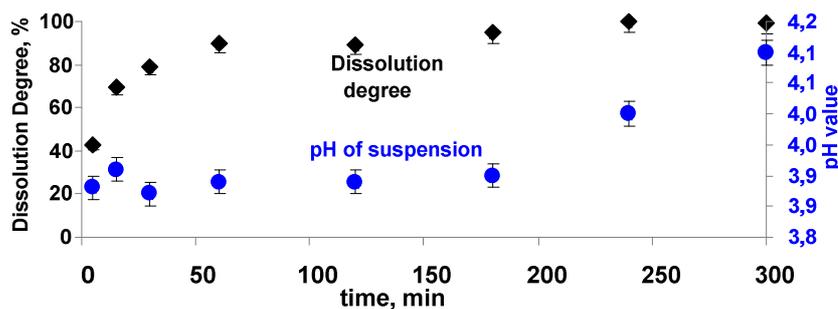


Fig.2. Dissolution degree of Zn nanoparticles and pH of suspension

N°P3a-31

PARTICULATE COBALT TOXICITY AND INTERNALIZATION IN LUNG CELLS

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Cobalt is widely used in the industry as it is included in the production of drying agents, pigments, and catalysts, and it is a major constituent of hard metal alloys. Radioactive isotopes of cobalt are also used in industry, medicine and nuclear research. In nuclear power plants, ⁵⁹Co-containing alloys can be activated into radioactive ⁶⁰Co oxides, dispersed in the cooling water, and represent a major concern. Occupational exposure to Co occurs mainly via inhalation leading to various lung diseases, such as pneumonitis, fibrosis and asthma. Although the chemical toxicity of cobalt has been proven, the molecular mechanisms of its toxicity are not well described and *in vitro* toxicity studies are mainly focused on cobalt chloride. As the risk of exposure to particulate cobalt oxide is a main industrial concern, we compared the toxicity of soluble chloride cobalt and oxide cobalt in *in vitro* tests. Since the lung is the main target organ of cobalt toxicity, we made this comparison using the human BEAS-2B lung cell line. First, we characterized the size and aggregation of particles using SEM, TEM, Specific Surface Area determination, and Dynamic Light Scattering. We followed the particle internalization pathway by TEM and also by using FACS (cell side scatter increase). By means of specific drugs, we confirmed the particle internalization route hypothesized from the TEM results. The cellular toxicity tests that we performed for particulate cobalt evidenced interferences. We proposed several assays to characterize these interferences in *in-vitro* toxicity tests and cell biology tests using flow cytometry (cell cycle analysis). These interference assays can be realized, in principle, whatever the nanoparticle considered. Interestingly, we successfully adapted the protocol of the CellTiter-Glo assay circumventing interference from cobalt oxide particles. With this new protocol, we have shown that the toxicity level (CI50) is consistent with the results obtained using the clonogenic assay which can be considered as a reference test, while the unmodified protocol predicted a toxicity ten times higher.

Our results show that: i) cobalt particles are much less toxic than soluble cobalt in our assay conditions, and ii) misleading conclusion may be drawn from toxicity testing of particles if interferences are not appropriately checked.

N°P3a-32

**EFFECTS OF PHYSICOCHEMICAL PROPERTIES, EXPOSURE DOSES AND CELL TYPES ON
CYTOTOXICITY OF ZINC OXIDE NANOPARTICLES**

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The increasing use of zinc oxide nanoparticles (nano-ZnO) has raised concerns about their environmental risks and potential hazards to human and environmental health. In this study, the toxicity of two OECD-sponsored commercial nano-ZnO products (Z-COTE (uncoated) and Z-COTE HP1 (coated with triethoxycaprylylsilane)) was investigated. Fine ZnO was also examined as a non-nanoparticle control. ICP-MS results indicated that the zinc content of Z-COTE, Z-COTE HP1 and fine ZnO was 99.8%, 98.5%, and 97.1%, respectively. TEM, SEM and AFM images showed that all samples were highly agglomerated, and the individual particles were crystalline rods or polygonal in shape. There was no significant difference in size distribution or average diameter of Z-COTE (53.5±22.5 nm) and Z-COTE HP1 (60.1±26.4 nm), but the fine ZnO was larger (106.9±41.1 nm). Three types of mouse cell lines, lung epithelial cells (FE1-MML cells), monocytes (RAW 264.7), and lymphoblasts (LBRM33), were exposed to 0, 3.125, 6.25, 12.5, 25, and 50 µg/mL of nano-ZnO for 24 hours. Dose-dependent cytotoxicity (altered cell morphology, trypan blue viability, cellular metabolic activity) was observed in all three cell lines, with the monocytic cells (LD50: 6.25-12.5 µg/mL) being more sensitive than lung epithelial cells (12.5-25 µg/mL) and lymphoblast cells (12.5-25 µg/mL). However, there were no significant differences in the cytotoxicity of Z-COTE, Z-COTE HP1 and fine ZnO towards each cell line. These results demonstrated that nano-ZnO had toxic effects on mammalian cells, and this effect was dependent on the exposure dose of ZnO, rather than the size and surface coating of particles. It was also found that the cell type can have a significant role in the cytotoxicity of nano-ZnO, which has implications for the safe and high-yield design of nano-ZnO for different applications.

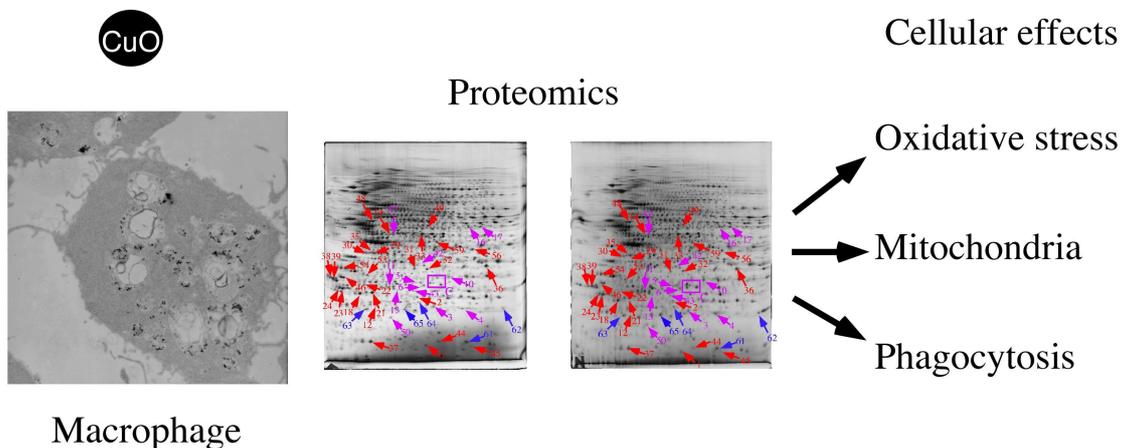
N°P3a-33

**PROTEOMIC STUDY OF THE MOLECULAR RESPONSES OF MOUSE MACROPHAGES TO
COPPER OXIDE NANOPARTICLES**

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The study presented here proposes molecular mechanisms of the responses of murine macrophages to copper oxide nanoparticles. To this purpose, proteomic analyses by two-dimensional electrophoresis/mass spectrometry have been used, using the RAW264.7 cell line as a model. Proteomics has pointed out several proteins which expression is changed when cells are treated with the copper oxide nanoparticles, suggesting alterations in the oxidative response (mostly peroxiredoxins and glutathione-based stress response), in the actomyosin cytoskeleton and in mitochondrial proteins, especially oxidative phosphorylation complexes subunits.

Functional analyses, carried out on both the RAW264.7 cells and on primary macrophages derived from bone marrow, have confirmed these results by showing a decrease in reduced glutathione levels, a decrease in the mitochondrial transmembrane potential, as well as an inhibition of phagocytosis and an inhibition of the lipopolysaccharide-induced nitric oxide production. Both proteomics and functional analysis also showed that part of the effects, but not all of them, can be attributed to the copper ion released from the nanoparticles, either directly in the culture medium or intracellularly after nanoparticles phagocytosis. Besides this molecular mechanisms, this study showed that macrophages are deeply altered, on a functional point of view, by copper-based nanoparticles.



N°P3a-34

TOXICOLOGICAL EVALUATION OF TiC NANOPARTICLES ORALLY ADMINISTERED IN A RAT MODEL

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* Both authors have participated equally in the study reported here.

Background: Titanium carbide (TiC) presents noteworthy properties, interesting for the fabrication of tool bits. However, there are very few studies in regard to the toxicological potential of TiC NPs.

Objectives: Study the toxicity and biodistribution of titanium carbide (TiC) nanoparticles in an *in vivo* rat model after acute (24 hours) and subacute (28 days) oral administrations. The acute doses were 0.5, 5, 50, 300 and 1000 mg·kg⁻¹, while the subacute doses were 0.5 and 50 mg·kg⁻¹.

Results: TiC biodistribution and elemental composition of feces and organs have been studied by Particle-Induced X-ray Emission (PIXE). Histopathological sections from organs (stomach, intestines, liver and kidneys) indicate the absence of damage at all applied doses, in both assessments. No alterations in the urine parameters (sodium, potassium, osmolarity) were found. TiC NPs were observed in urine.

Conclusion: This is the first study that assesses the toxicity, biodistribution, and composition changes in feces and organs of TiC nanoparticles in an *in vivo* rat model. TiC was excreted mostly in feces and low traces were retrieved in urine, indicating that TiC can cross the intestinal barrier. No sign of toxicity was however found after oral administration.

Keywords

TiC nanoparticle, plasma, biodistribution, PIXE, toxicity

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N°P3a-35

IN VITRO TOXICITY ASSESSMENT OF GOLD NANOPARTICLES IN BIOLOGICAL MEDIA

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Potential applications of gold nanoparticles (GNPs) are increasing in the sector of biomedicine; from chemical detection and bio-imaging towards therapy [1]. Efficient conversion of strongly absorbed light by plasmonic GNPs to heat energy and their easy bioconjugation suggest their use as selective photothermal agents in targeted cancer cell killing [2]. Studies have revealed that the same properties that make nanoparticles so unique could also be responsible for their potential toxicity to non-cancerous or healthy cells [3, 4]. But these studies are not sufficient; considering their surface chemistry and interaction with the cell membranes as this interaction can disrupt native conformation of membrane proteins and cause cell death by ceasing functionality. Moreover, to meet the demand of high throughput biological applications, newer GNPs are being fabricated that may create fatal effect to biological environment.

The aim of this work is to investigate the effects of different parameters of GNPs on cell viability to determine the less toxic form of these nanoparticles to use in the cancer therapy like photothermal therapy. In this study we report the comparative *in vitro* assessment of GNPs cytotoxicity (already being used & newly fabricated) on human cell line based on different parameters. Two different sizes (15 & 50 nm) and shapes (spheres and flowers) of GNPs with two different surface chemistries have been studied by varying their concentration. Human myelogenous leukemia cell K562 and Human Umbilical Vein Endothelial Cell (HUVEC) were incubated in presence of these particles. It is found that gold nanoflowers (GNF) show more toxicity than the gold nanospheres (GNS) even at lower concentrations (below picomolar concentration). But PEGylated surface alters and reduces this toxic effect. GNPs internalize inside cells in short duration of NP-cell incubation (<3hrs) and aggregation is observed in the cytoplasm near the periphery of the nucleus. Considering both viability and proliferation rate, GNS show more biocompatibility than the GNF during the *in vitro* assessment with the cells.

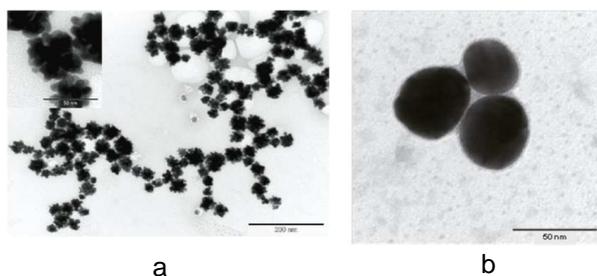


Figure: TEM images of gold nanoparticles used for this toxicity study;
a) gold nanoflower, b) gold nanosphere [5]

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N°P3a-36

COMPARISON CYTOTOXIC POTENCY OF ZINC OXIDE NANOPARTICLES ON FIVE CELLULAR LINES.

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Zinc oxide nanoparticles are currently the most commercially important nanomaterials produced on a large scale. They are mainly used in cosmetics industry (as an ingredient for skincare products, sun creams), as an additive in construction industry (paints, coatings), as an optoelectronic material and many others. Toxicological data on the toxicity of zinc oxide nanoparticles are inconclusive. The aim of this study was to assess the cytotoxic potency of zinc oxide nanoparticles depending on their particle size and tested cells.

Cytotoxicity of zinc oxide (ZnO) nanopowders (< 50 nm; and <100 nm particle size) and metallic zinc oxide powder (> 2.5 µm) was evaluated on five cellular lines: Chinese hamster ovary cells (CHO), mouse testicular Sertoli cells (15P-1), human pulmonary cancer cells (A549), mouse macrophage-like RAW 264.7 cells and human epithelial carcinoma cells (A431), after 24-hour exposure. The MTT reduction assay, which assesses the metabolic activity of cells and NRU (Neutral Red Uptake) assay measuring cell membrane permeability were used. The IC₅₀ values (i.e. the concentration required for a 50% cell density/viability reduction) were used as the main measure for comparing the cytotoxicities of the tested compounds. Additionally the analysis of the level of cellular thiols was carried out.

All tested zinc oxide particles showed a dose-dependent cytotoxicity. The most toxic compound was ZnO < 100 nm, but it was only slightly more toxic than metallic zinc oxide (> 2.5 µm). ZnO nanoparticles < 50 nm showed the weakest cytotoxicity in all tests conducted on all cells. There were great differences in the reactivity of tested cells to the zinc oxide particles. The skin cells (A431) did not show statistically significant differences in toxicity of compounds, additionally the IC₅₀ values were the highest for all compounds. Similarly high IC₅₀ values were obtained in the case of A549 cells. The reproductive system cells (15P-1 then CHO) were the most sensitive experimental model for testing the overall cytotoxic effect of tested compounds. The IC₅₀ values obtained suggest that MTT assay was the most sensitive assay in all the cell lines.

This paper has been based on the results of a research task carried out within the scope of the second stage of the National Programme "Improvement of safety and working conditions" partly supported in 2011–2013 — within the scope of research and development — by the Ministry of Science and Higher Education/National Centre for Research and Development. The Central Institute for Labour Protection – National Research Institute is the Programme's main co-ordinator.

N°P3a-37

RESPIRATORY EFFECTS OF REPEATED INSTILLATIONS OF IRON, MANGANESE, AND CHROMIUM OXIDES NANOPARTICLES IN MICE

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Introduction: Nanoparticles (NP) are widely used in different domain (utensils, food, medical...) which raises questions about their toxicity on Human beings. Because of the presence of NP in welding fumes, welders represent a human model of exposure to NP. They can develop different pulmonary pathologies such as chronic bronchitis, fibrosis and cancer. In order to evaluate the potential role of NP from welding fumes in the development of pulmonary diseases observed in welders, the aim of our study is to examine the respiratory effects of repeated instillations of metal oxides NP, representative of those found in welding fumes, in mice.

Methods: Once a week, 5µg of NP of Fe₂O₃, Fe₃O₄, MnFe₂O₄, CrOOH NP or the mix of the 4NP was oro-pharyngeally administrated to C57/Bl6 mice during 1day, 1month and 3months. Another group of mice were instilled with 20µg of NP, corresponding to the total dose received by mice during 1month (4x5µg). At the time of the sacrifice, we performed a bronchoalveolar lavage (BAL). The lungs, the spleen, the kidneys and the blood were sampled. Pulmonary inflammation was studied in the BAL and the lungs by: analysis of total cellularity, protein and cytokines content by Bradford and ELISA (IL-1β, TNF-α, TGF-β) and qRT-PCR. Oxidative stress, the presence of NP and histopathological changes were studied in the lungs respectively by qRT-PCR and immunostaining for HO-1, X-ray microfluorescence (XRF) and optical microscopy (OM) analysis.

Results: The BAL cellularity of mice repeatedly instilled during 1 and 3months with Fe₃O₄, MnFe₂O₄ NP significantly increased compared to control mice. Besides, total protein content in the BAL of the mice repeatedly instilled with MnFe₂O₄ NP during 1month significantly increased compared to control mice. No significant variation of the different cytokines level in the BAL was observed regardless to the time of instillation and the nature of the NP. Clusters of particles in the lungs of mice repeatedly instilled with Fe₃O₄, MnFe₂O₄ NP during 1month and with 20µg of MnFe₂O₄ NP were observed by XRF and OM although, no histological changes were observed. The analysis of the expression of inflammation and oxidative stress markers is under investigation.

Conclusion: The repeated instillations of low doses of NP didn't induce severe effects in view of the cytokines secretion in BAL and the absence of histological changes in lungs. Yet, the particles clusters present in the lung may induce late effects depending on their persistence in mice lungs.

N°P3a-38

**MORPHOLOGICAL AND CYTOHISTOCHEMICAL EVALUATION OF RENAL EFFECTS OF
CADMIUM-DOPED SILICA NANOPARTICLES GIVEN INTRATRACHEALLY TO RAT.**

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The proposed applications of silica/cadmium containing nanomaterials in medicine and technology have attracted much attention in the latest years. However, information on toxicological and health impact of these nanomaterials is still limited. Our recent studies on model cadmium-containing silica nanoparticles (Cd-SiNPs) indicated long-lasting gene expression changes occurring in kidney after intratracheal instillation (i.t.) of these nanoparticles in rats (J. Nanopart. Res. 2012, 14:925). Compared with CdCl₂ the effect of Cd-SiNPs was more selective. Furthermore, delayed gene expression alterations linked to apoptosis and regulatory processes were observed 30 days post-treatment in rats given Cd-SiNPs but not in those receiving CdCl₂.

In this work, cyto-histochemical methods were used to examine in rats renal responses to i.t. application of Cd-SiNPs and determine whether morphological and biochemical parameters were modified by these nanoparticles in association with toxicogenomic changes.

Groups of Sprague Dawley rats were intratracheally instilled with a single dose of Cd-SiNPs (1 mg/rat), CdCl₂ (400 µg/rat), SiNPs (600 µg/rat), or 0.1 ml saline (control). Renal effects, evaluated 7 and 30 days post-exposure, included (i) histopathology of renal tissue (Haematoxylin/Eosin Staining and TEM analysis) and (ii) characterization of apoptotic/proliferating features by TUNEL and PCNA immunostaining.

Area-specific renal cell apoptosis was observed in all treatment groups, the cortex and inner medulla being the most affected regions. The apoptotic changes were apparent already at 7 days post-exposure in both affected areas, and were still observable in inner medulla 30 days after treatment. The increase in apoptotic frequency was more pronounced in Cd-SiNP-treated animals compared to either CdCl₂ or SiNPs groups. Histological findings showed comparable alterations in the renal glomerular (cortex) architecture occurring in all treatment groups at both time-points considered. The glomeruli appeared often collapsed, showing condensed, packed mesangial and endothelial cells. Oedematous haemorrhagic glomeruli were also observed in Cd-SiNPs-treated animals, indicating involvement of the vascular component in the renal mesangium, with numerous capillary loops distorted.

I.t. administration of bare SiNPs caused morphological and apoptotic changes but did not modify the gene expression profile in the kidney.

These findings support the concept that multiple assays and an integrated testing strategy should be recommended to characterize toxicological responses to nanoparticles in mammalian systems (Grants from: Italian Ministries of Health, Research, Education; and Cariplo Rif 2011-2096).

N°P3a-39

**TITANIUM OXIDE NANOPARTICLES TOXICITY CAUSES FUNCTIONALITY AND DNA DAMAGE
IN BUFFALO (BUBALUS BUBALIS) SPERM *IN VITRO*.**

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With the growth of nanotechnology, there is tremendous increase in the applications of nanoparticles (NPs) and nanomaterials in the daily used products and appliances, which concomitantly increases the risk of human as well as animal exposure to these nanomaterials through various routes. Their unique physico-chemical properties due to the high surface to volume ratio of these particles could produce these unpredictable effects. The present study has examined the effect of different concentrations (1 µg/ml, 10 µg/ml and 100 µg/ml) of titanium oxide (TiO₂) nanoparticles (NPs) (<100nm) on viability, membrane integrity, capacitation status and DNA integrity of buffalo spermatozoa. Characterisation of nanoparticles was done by the transmission electron microscopy (TEM) and dynamic light scattering (DLS). Sperm chromatin dispersion (SCD) test and acridine orange test (AOT) were employed to detect DNA fragmentation in sperm treated with NPs. There was significant (P<0.05) decrease in cell viability and membrane integrity (assessed by enzyme leakage) at 6 hours incubation with NPs. However, significant (P<0.05) increase in sperm capacitation was observed for TiO₂ NP albeit at lower concentrations. In DNA fragmentation assay, there was dose dependent increase in the DNA fragmentation (r=0.96). Ultra-thin cross sections revealed TiO₂ NPs inside head and plasma membrane of the buffalo spermatozoa as assessed by TEM. Overall enzyme assay for membrane integrity suggested that TiO₂ NPs causes damage to cell and acrosomal membrane and mitochondria, leaking the alkaline phosphatases, hyaluronoglucosaminidase, succinic dehydrogenase and 5' Nucleotidase. There was decreased in membrane integrity after 6 hours of incubation at higher concentration of TiO₂ NPs also observed by a simple test (HOST). Nevertheless there was increase in capacitation and acrosomal reaction with increase in dose of NPs. The reason behind increase in % capacitation and acrosome reaction is not known. But herein we hypothesised, from low (1 µg/ml and 10 µg/ml) to high (100 µg/ml) concentration of TiO₂ NP, the increasing concentrations were sufficient to interact with sperm membrane in a specific manner, destabilizing the membrane, facilitating increasing in membrane fluidity, leading to increase in capacitation followed by acrosome reaction. These studies suggest that TiO₂ NPs may have cytotoxic effect on buffalo spermatozoa by affecting sperm functionality and causing high amount of DNA fragmentations.

N°P3a-40

**CYTOTOXICITY AND GENOTOXICITY OF ZINC OXIDE NANOPARTICLES IN HUMAN
NEUROBLASTOMA CELLS**

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Nanotechnology has rapidly grown in recent years, but the pace of development has not been matched by a complete investigation of their safety. Zinc oxide nanoparticles (NPs) are of the more frequently used type of nanomaterials with wide applications that range from electronic usage to biomedical interventions. In this study, the effects of these NPs were evaluated in human SHSY5Y neuroblastoma cells. These NPs were characterized for size and dispersion (both in water and media) before carrying out genotoxic and cytotoxic tests; possible cellular uptake was also determined.

Data obtained showed that, despite they are not able to enter in cells, ZnO NPs induce both cytotoxicity and genotoxicity. Cytotoxicity was evaluated with MTT and NRU assays and genotoxicity with Comet assay, Micronucleus assay and γ H2AX assay. In addition, ZnO NPs were also able to alter cell cycle and to induce oxidative damage (assessed by Comet assay) These results provide relevant information on the possible risks of exposure to these NPs in terms of human health and indicate that nanoparticles do not have to enter human neuroblastoma cells to alter their normal function.

We gratefully acknowledge the support provided by the Nano LINEN project, funded by the New Indigo Program.

N°P3a-41

INNOVATIVE NANO-QSAR TECHNOLOGY FOR EARLY DETECTION OF NANOPARTICLES' TOXICITY

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Understanding the relationships between physicochemical properties of nanomaterials' and their interactions with biological systems is a major challenge to the development of nanotechnology. Quantitative structure-activity relationship (QSAR) models were developed to define mathematical relationships between measured properties of nanoparticles and their structure.

The aim of the current study is implementation of improved nano-QSAR technique in order to create a tool for early detection of biological fates of nanoparticles.

Innovative nano-QSAR technology is based on the Simplex representation of molecular structure (SiRMS), the fractal theory, cluster theory and crystal structure data for the description of nanoparticle clusters.

In SiRMS method each compound is represented as a system of different simplexes (tetraatomic fragments of fixed composition, structure and symmetry). The structure of each simplex in molecule has been determined considering differences in atom type and bond types. All atoms in a simplex are differentiated, based on such characteristics as element, partial charge, lipophilicity, atomic refraction, electronegativity of atom, donor/acceptor of H-bond, etc.

In addition to SiRMS descriptors crystal structure parameters were computed. Some of crystal descriptors were calculated by SiRMS method.

Surface topology was simulated by fractal descriptors. Selected fractal descriptors depend on the size of studied nanoparticles. Such characteristics of nanoparticles as shape, square, volume, etc., were computed by special cluster descriptors.

In this research the cytotoxicity of 17 different types of metal oxide nanoparticles to bacteria *Escherichia coli* and 11 different types of nanoparticulate materials to murine macrophage cell line were described as an example of this approach. All original experimental data have been taken from the literature.

QSAR tasks have been solved using the PLS regression. Statistic characteristics of models are quite satisfactory: squared regression coefficient (R_2)>0.85; cross-validated regression coefficient (Q_2)>0.75, root-mean-square error of cross-validation (RMSE)<0.3. Best results based on a consensus of multiple QSAR models were generated. Calculation results were found to be consistent with experimental data.

The high impact of polarizability, crystal structure parameters and atom individuality on toxicity of investigated nanoparticles was determined.

It was shown that novel nano-QSPR technology for nanoobjects is helpful for biological properties' investigation.

N°P3a-42

IN VITRO TOXICITY OF CARBON NANOTUBES: IMPACT OF ACID FUNCTIONALIZATION

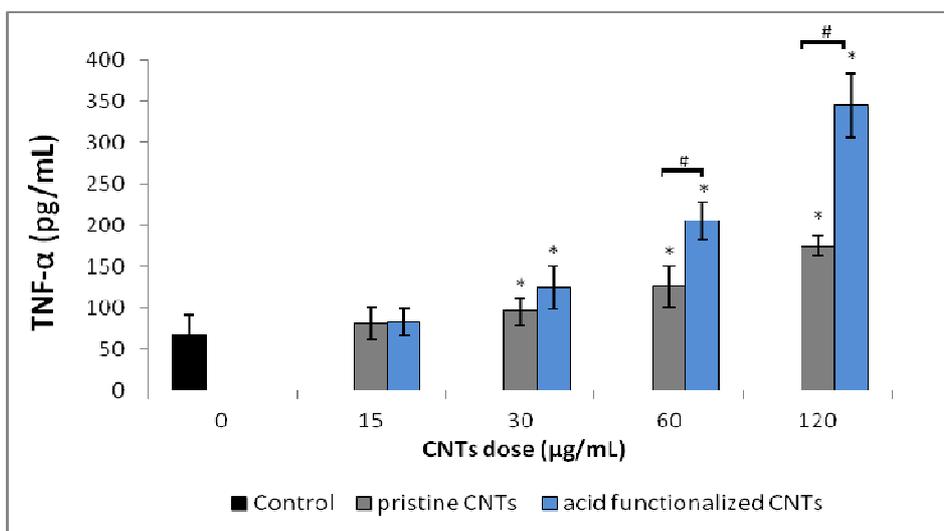
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With a small aerodynamic radius, carbon nanotubes (CNTs) have the potential to access deeply into the lung and activate an immune response from the alveolar macrophages. The mechanisms involved are believed to depend on their physicochemical features. This study assesses the impact of an acid functionalization of the CNTs, on the *in vitro* cellular response of a mouse leukemic macrophage cell line (RAW 264.7).

A full physicochemical characterisation was conducted on pristine (raw surface) and carboxyl functionalized multi-walled carbon nanotubes (pCNTs and fCNTs respectively). The presence of -COOH functional groups was first confirmed by thermal desorption and Raman spectroscopy. The acid functionalization process was thought to attack the tube surface as a slight decrease in diameter was observed. A small increase in specific surface area likely due to surface defects and a strong reduction of catalytic impurities corroborated this assumption. Lastly, carboxyl groups on the surface decrease the hydrophobic nature of pristine CNTs enabling a better dispersion in water or culture medium observed by DLS (Dynamic Light Scattering) after optimised sonication.

In vitro experiments have been carried out to evaluate biological toxicity with different outcomes: cytotoxicity, inflammation and oxidative stress. Membrane integrity was not compromised after 24h of contact with 15 to 120 µg/mL pCNTs and fCNTs: LDH (lactate dehydrogenase) leakages were detected at the same level as for cells alone. Microscopic observations showed however that cells interact with CNTs mainly through phagocytosis. With a dose as small as 15µg/mL CNTs, cells were activated, presenting numerous vacuoles, some of them containing CNTs agglomerates. At higher concentrations cells were saturated by CNTs and morphological damages were observed. A short contact of 90min with CNTs induced oxidative stress with a tendency of higher production of reactive oxygen species for pCNTs than fCNTs (about 1/3 more). Inflammation, assessed by TNF-α (Tumour Necrosis Factor-α) production, was increased by both CNTs in a dose-dependent manner, but at significantly higher levels for elevated concentrations of fCNTs.

In conclusion, acid functionalization seems to protect against oxidative stress likely through the reduction of catalytic impurities, but increases inflammation of RAW macrophages thus indicating a different cellular response. This confirms the importance of assessing physicochemical characteristics especially surface chemistry.



Inflammation after 24h contact with pristine and acid functionalized carbon nanotubes ($p < 0.05$, Student's test).

N°P3a-43

ROLE OF METAL OXIDE NANOPARTICLE IN WELDER'S LUNG INJURY

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Increasing concern exists regarding possible human toxicity of manufactured nanoparticles (NP). So far, there is almost no study assessing the consequences of human exposure to NP, including respiratory toxicity. This point is critical as regard to risk evaluation for human health. Our original approach using welders as a model population of human respiratory exposure to NP. Lung tissues from 21 welders exposed to welding fumes during at least 10 years were compared to 21 unexposed patients matched on age, asbestos exposure and tobacco smoking. Pulmonary lesions were investigated by optical microscopy on lung tissue section (Hematoxylin-Eosin-Safran and Perls staining). We observed an overload of iron (siderophages and ferruginous bodies) and a severe peribronchiolar and perivascular fibrosis significantly higher in welders compared to unexposed patients. To localize NP in the lung tissue and determine NP form, chemical composition and size, X-ray microfluorescence (μ XRF) and Scanning transmission electron microscopy (STEM)-Energy dispersive X-ray (EDX) was used on lung tissue sections. μ XRF allowed the detection of excessive Fe, Cr and Mn mainly inside macrophages, and co-localized within fibrosis lesions in welders, which have been identified as metal oxide NP by STEM-EDX.

In this context, we set-up an *in vitro* study, with the hypothesis that NP could initiate an inflammatory process that would participate in the lung changes observed in welders. Human macrophages (THP-1 cell line) were exposed for 24 h to NP representative of those found in welders: Fe_2O_3 , Fe_3O_4 , MnFe_2O_4 and CrOOH (diameter 25 nm). No cytotoxicity was observed up to $25 \mu\text{g}\cdot\text{cm}^{-2}$ by MTT and LDH assay. Exposure media were analyzed by Luminex and showed that Fe_2O_3 , MnFe_2O_4 and CrOOH NP induced the secretion of cytokines IL-1 β , IL-6, IL-10 and TNF- α , and chemokines CXCL8, CCL2, CCL3, CCL4, CCL-7 and CCL-22. This chemokine secretion was associated with the induction of macrophage migration (observed in Boyden chambers). No secretion of IL-4, IL-5, IL-12, IL-13 was detected. The Fe_3O_4 NP only induced an increased secretion of IL-1 β and chemokines CXCL8 and CCL7, indicating that the effects of NP on macrophages' secretome are dependent on physicochemical characteristics of NP.

In this study, we showed that exposure of macrophages to metal oxide NP representative of welding fumes, induced an essentially M1 phenotype, with the secretion of proinflammatory cytokines and chemokines which could induce migration of macrophages on the site of exposure. This suggests that NP could have an important role in lung changes observed in welders and therefore could have further consequences on respiratory health.

N°P3a-44

MTT AND LDH INTERLABORATORY ASSAYS FOR ASSESSING IN VITRO CYTOTOXICITY OF ENGINEERED NANOMATERIALS

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Most of the assays used for toxicity assessment were designed and standardized for chemical toxicity and are now used for evaluation of toxicity of engineered nanoparticles and rather different observations were published. This could be attributed to nanoparticles which present unique physicochemical properties that can interfere with the use of classical toxicity assays. Therefore validation studies of these techniques to assess *in vitro* cytotoxicity of engineered nanomaterials appear necessary. They would make possible the achievement of standardized protocols and enable to compare results coming from different studies. Developing such validation step would demonstrate reliability, transferability, precision, accuracy and relevance of the assay.

In the interlaboratory study presented here, MTT and LDH assays were performed using two different titanium dioxide (TiO₂) nanoparticles suspensions prepared by the same dispersion procedure and human SH-SY5Y neuroblastoma cells (all with the same origin). Prior to testing of these NPs, they were characterized for their size, agglomeration status, zeta potential, crystal phase, specific surface area. Each laboratory, for both assays, tested five different concentrations of each nanoparticle in addition to negative and positive controls and four periods of exposure (3, 6, 24 and 48h). Same trends are observed in various laboratories even if the results could not be directly compared in terms of absolute value: almost no mortality of cells till 48 hours of exposure from MTT tests while LDH leakage appears at low nanoparticles concentrations. Data showed first a size effect of the Nps and second that MTT assay results present higher variability between laboratories than LDH results. These results from this study provide valuable information on the validity of MTT and LDH assays to assess cytotoxicity of engineered nanoparticles.

N°P3a-45

UNDERSTANDING THE IMPACT OF NP PHYSICOCHEMICAL PROPERTIES IN BIOLOGICAL FLUID AND THEIR RESULTANT CELLULAR INTERACTION *IN VITRO*

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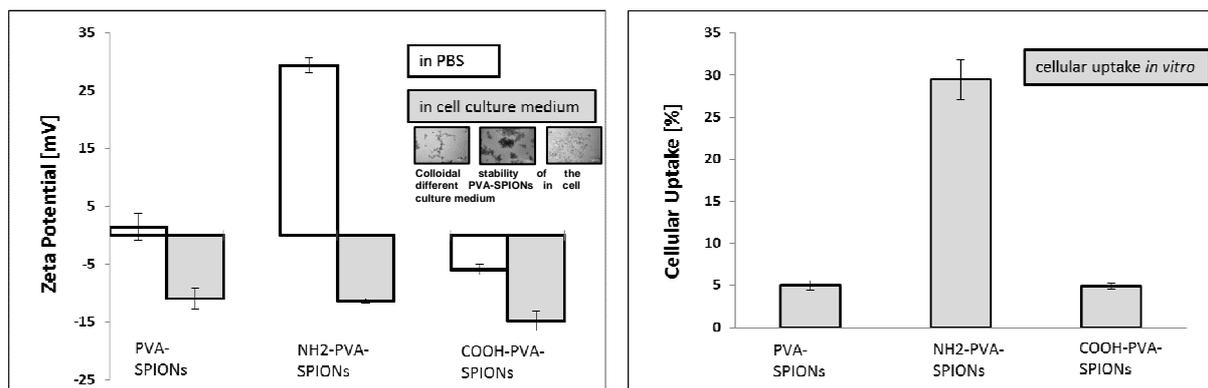
The cellular interaction of nanoparticles (NPs) strongly depends upon their physicochemical properties (e.g. material, size, shape and surface charge)¹⁻⁴. In particular, the surface charge of NPs is known to be one of the most essential factors that directly relates to their cellular uptake.^{5,6} In addition, the colloidal behaviour of NPs is influenced by their environment (e.g. medium density and viscosity).

In the present study, we show how nanoparticle-protein interactions relate to the particular physicochemical characteristics of the particles, such as their colloidal stability, and how this significantly influences the subsequent nanoparticle-cell interaction *in vitro*.

Therefore, different surface charged polyvinyl alcohol (PVA) coated superparamagnetic iron oxide NPs (SPIONs) were synthesized and characterized. After incubation with fetal bovine serum, sodium dodecyl sulphate polyacrylamide gel electrophoresis (1D SDS-PAGE) and mass spectrometry techniques (LC-MS/MS) identified very similar adsorbed protein profiles, independent of the surface charge of the particles.

Despite the comparable protein corona, which was also confirmed by similar zeta potential values after serum incubation, cellular uptake of positively charged PVA-SPIONs was quicker and significantly higher compared to both neutral PVA-SPIONs and negatively charged PVA-SPIONs. However, static and dynamic light scattering (SLS and DLS) measurements within the biological fluid showed that positively charged nanoparticles displayed a significantly lower colloidal stability than neutral and negatively charged particles, leading to a higher non-sedimentation driven cell-internalization *in vitro* without any significant cytotoxic effect.

The results of this study therefore strongly indicate that upon considering the human interaction of NPs it is of the uppermost importance to first understand how NPs interact with their surrounding biological fluid, before investigating as well as interpreting responses and effects of NPs at a cellular or *in vivo* level.



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N°3a-46

BIOCOMPATIBILITY EVALUATION OF MEDICAL DEVICES INCORPORATING NANOMATERIALS

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The primary objective of the ISO 10993 standards governing the biological evaluation of medical devices is the protection of humans from potential biological risks arising from their use. These standards are intended for helping manufacturers anticipate bio-incompatibility of their medical devices. On the basis of chemistry and/or biocompatibility testing, associated with literature data, clinical data, etc, the safety of classical medical devices can be established.

Regarding medical devices incorporating nanotechnologies, the validation of their biocompatibility is much more complex due to i) very limited toxicological data nowadays available on nanoscale raw materials which properties generally differ from those of their bulk counterparts, ii) the absence of relevant control materials, iii) the large number of physicochemical parameters that will influence the toxicity of nanomaterials, iv) the necessity to adapt some standardized protocols to avoid false positive responses, etc.

Therefore, case-by-case adaptations are required to take into account the specific features of nanomaterials. Some practical examples issued from biocompatibility evaluation of nanodevices will be provided.

N°P3a-47

GENOTOXICITY AND PROINFLAMMATORY EFFECTS OF NANOSILICA VIA ORAL ROUTE: *IN VITRO* AND *IN VIVO* APPROACHES

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Silica (SiO₂) in its nanosized form is now used in food applications although the potential risks for human health need to be evaluated.

In this study, the genotoxicity and the pro-inflammatory effects of four different types of amorphous silica were investigated both *in vitro* and *in vivo*.

In vitro experiments were conducted in the human intestinal Caco-2 cell line exposed for 24 h. After cytotoxicity assays with XTT and neutral red uptake, chromosomal damage were identified using the cytochalasin block micronucleus assay along with the release of the proinflammatory interleukin 8 (IL-8) in the culture medium.

For *in vivo* experiments, male Sprague Dawley rats were administrated 5, 10 or 20 mg/kg bw/day for three days by gavage. DNA damage in six organs as well as in blood were measured with the alkaline comet assay. Formamidopyrimidine DNA glycosylase (Fpg)-modified comet assay was also used to detect oxidative DNA damage.

Results did not show an obvious genotoxic response either *in vitro* or *in vivo* irrespective of the organ or the nanoparticle. It should be noted that the responses varied largely between experiments and animals. However the level of IL-8 secretion *in vitro* was increased with two out of the four silica nanoparticles. Our results underline the difficulty for accurate hazard evaluation related to nanoparticles.

N°P3a-48

**IN VITRO EVALUATION OF CELLULAR RESPONSE INDUCED BY
ZNO NANOPARTICLES, ZINC IONS AND NON-NANO ZNO IN FISH CELLS.**

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Zinc Oxide nanoparticles (ZnO-NP) are abundantly produced because of its high stability, photocatalytic and anticorrosion property. ZnO-NP is inevitably released in the environment representing a possible danger to aquatic life. Their toxic potential using in vitro systems will allow the explanation of their potential interaction with cellular biomolecules. Clarification of such mechanisms is important given that source and properties of the cells could influence the toxic response.

The aim of this study was to evaluate cytotoxic potential of ZnO-NP in Rainbow trout gonad (RTG-2), Rainbow trout liver (RTL-W1) and Rainbow trout hepatoma (RTH-149) cells, by comparison with two reference toxicants, zinc ions and non-nano ZnO. Different aspects of cellular activity were tested, including Alamar Blue (AB) assay for oxide reductase enzyme activity, 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) assay for membrane function, β -galactosidase (β -gal) assay for cellular stress, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for the mitochondrial electron transport chain and kenacid blue protein (KBP) assay for cell growth. Intracellular reactive oxygen species (ROS) generation, glutathione (GSH) content, glutathione S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) activities were also evaluated. Chemical exposure was conducted for 6, 24 and 48 h on each of the three cell lines.

The ZnO-NP suspensions were characterised using dynamic light scattering (DLS) and Transmission Electron Microscopy (TEM). In order to investigate the effect of zinc ions on cell viability, stock ZnO-NP suspension was kept on a shaker overnight, and then centrifuged and filtered through an alumina Whatman Anotop filter 0.02 μ m.

The results in all three cell lines suggested that the highest concentration of ZnO-NP, zinc ions and non-nano ZnO (100 μ g/ml) did not produce a significant effect of cell growth (KBP assay) or oxide reductase enzyme activity (AB assay) over 6h, 24h or 48h exposures. Treatment with ZnO-NP, zinc ions and non-nano ZnO, in all three cell lines, induced damage to the cell membrane over 24h or 48h exposure (CFDA-AM assay), elevated levels of oxidative stress over 6h, 24h or 48h exposure (β -gal and ROS assay), glutathione content depletion over 6h, 24h or 48h exposure (GSH assay), glutathione reductase and glutathione peroxidase decrease activities over 6h, 24h or 48h exposure (GP and GPx assays) and increase of glutathione S-transferase level over 6h, 24h or 48h exposure (GST assay). The results demonstrate that RTH-149 cells are more sensitive than RTG-2 and RTI-W1 to the toxicity of ZnO-NP, zinc ions and non-nano ZnO. Toxicity experiments revealed comparable toxicity for ZnO-NP, zinc ions and non-nano ZnO, probably attributable solely to dissolved zinc rather than ZnO particle size.

This study was funded by the Spanish project RTA2010-00018-00-00.

N°P3a-49

GENOTOXICITY OF CERIUM DIOXIDE NANOPARTICLES (CeO₂ NPS) ON MICE OOCYTE.

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Introduction: Nanoparticles (NPs) are objects with at least one dimension between 1-100 nm with properties specifically resulting from this nanometric size. Cerium dioxide nanoparticles (CeO₂ NPs) are largely used in the automotive industry as additives in diesel fuel. According to the Health Effects Institute (HEI), CeO₂ NPs emissions are expected to reach up to 22 million pounds annually in the European Union after introduction into diesel-additive. Moreover, the Organization for Economic Cooperation and Development (OECD) has included CeO₂ NPs in the priority list of the nanomaterials requiring urgent evaluation. While the genotoxicity of CeO₂ NPs towards somatic cells has already been studied, only few studies focused on germ cells. We will present here the main results of one of the first research work focused on the genotoxic impacts of CeO₂ NPs towards oocytes. After a thorough characterization of the CeO₂ NPs, we assessed the internalization of CeO₂ NPs in mice oocytes and follicular cells *in vitro*, and their genotoxicity.

Material & Method: CeO₂ NPs are pseudo-spherical crystallites of cerianite with size of 2-3 nm. Hydrodynamic diameter of CeO₂ NPs in biological media were assessed by laser diffraction. The oocytes have been obtained by superovulation of CD1 mice. Follicular cells and oocytes with or without pellucid zona were incubated *in vitro* for 2 hours with 100mg/l CeO₂ NPs and the potential internalization has been studied by TEM (transmission electronic microscopy). The genotoxicity of CeO₂ NPs on oocytes and on follicular cells was studied at 4 concentrations (2, 5, 10 et 100 mg/l) using the comet assay (Berthelot-Ricou et al., 2011) in order to detect and quantify primary DNA lesions based on the analysis of the Olive Tail Moment (OTM = % DNA in the tail x length of the comet tail).

Results: Hydrodynamic diameter of CeO₂ NPs in biological media reached few microns. The TEM study showed the internalization of CeO₂ NPs aggregates by endocytosis in peri-oocyte follicular cells. Nevertheless, after incubation of CeO₂ NPs with decoronised oocytes, the CeO₂ NPs aggregates were only observed around the pellucid zona. After depellucidation, we did not observe NPs in the oocyte cytoplasm. Using the comet assay, significant DNA lesions were observed on follicular cells at the four concentrations tested. The genotoxic analysis of CeO₂ NPs on oocytes with zona pellucid induced a dose related significant increase in DNA lesions and the OTM values increases were statistically significant only at 10mg/l and 100 mg/l. Similar results were observed on oocytes without pellucid zona.

Conclusion: *In vitro* incubation with 2-100 mg/l CeO₂ NPs induced a genotoxic stress in either follicular cells or oocytes, although internalization process was evidenced in follicular cells only. For high CeO₂ NPs concentrations, we hypothesized that the oocyte DNA lesions could be induced by an oxidative stress mechanism occurring at the surface of the cells.

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N°P3a-50

EFFECTS OF AMORPHOUS SILICA NANOPARTICLES ON HUMAN ALVEOLAR EPITHELIAL CELLS

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Silica nanoparticles (SiO₂-NPs) have many applications and are exploited in various commercial products such as pharmaceuticals, cosmetics, food and in particular biomedical imaging by using fluorescently labelled SiO₂-NPs. To evaluate the adverse health risks of human exposure to SiO₂-NPs, it is essential to evaluate SiO₂-NPs toxicity especially on the respiratory system. In this study, we investigated the toxicological effects of SiO₂-NPs on the human alveolar epithelial cell line A549 and investigated the mechanisms involved in the cellular responses.

We used fluorescently labelled SiO₂-NPs (28 nm) to study their uptake by the human alveolar epithelial cell line A549 using confocal microscopy. To characterize the SiO₂-NPs physicochemical properties, we measured the aggregation state and the zeta potential in culture media as well as the intrinsic capacity of SiO₂-NPs to produce reactive oxygen species (ROS) by the DiThioTreitol (DTT) assay. The cytotoxicity of SiO₂-NPs was evaluated by the WST-1 assay after 24 hours of treatment at 2.5 to 40 µg/cm². Oxidative stress induced by SiO₂-NPs was assessed by measuring the induction of superoxide dismutase (SOD). The pro-inflammatory response was evaluated by measuring release of the chemokine IL-8 by the cells and IL-8 mRNA expression.

Our results show that, after 4 hours of treatment, SiO₂-NPs are taken up by human alveolar epithelial cells but are not localized within lysosomes as they do not colocalise with LAMP (lysosomal associated membrane protein) staining. SiO₂-NPs are cytotoxic in a dose-dependent manner. This cytotoxicity could be due to oxidative stress as SiO₂-NPs produced ROS in acellular conditions in a dose-dependent manner. However, we observed that expression of SOD2 was not modified in A549 cells in response to SiO₂-NPs exposure at non cytotoxic concentrations after 24 hours of treatment. SiO₂-NPs induced a pro-inflammatory response observed by an increase of IL-8 secretion and IL-8 mRNA expression.

In conclusion, 28 nm SiO₂-NPs are internalized by human alveolar epithelial cells but exposure results also in dose-dependent cytotoxicity which could be due to an oxidative stress induction. Moreover, SiO₂-NPs induce a pro-inflammatory response in A549 cells at non cytotoxic concentrations. Our study underlines the need to determine the toxic effects of NPs and the mechanisms involved in the biological responses of the respiratory epithelium for the safe use of nanoparticles.

Keywords: silica, nanoparticles, cytotoxicity, oxidative stress, pro-inflammatory response, internalization.

Supported by FP7 program n°201335 NanoTEST

N°P3a-51

HOW DO CARBIDE ENM DISPERSIONS EVOLVE IN AN *IN VITRO* ASSESSMENT?

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With the advent of the discovery of engineered nanomaterials (ENMs) and their fundamental different properties than bulk materials, there has been a rush in the industry to produce and use these new materials in different products, touching a wide area from semiconductors, medicine, products of personal use, and food. There are health concerns regarding the use of ENMs. It has been shown that the way ENMs are prepared for *in vitro* tests influences the toxicity results. The heterogeneity observed in the results from the potential toxicity assessment evaluation of nanoparticles, prevents their adequate comparison or evaluation. Several factors are reported to potentially influence the modification of their physicochemical properties during the different stages of the assessment (the pre-dispersion, the mixture with the culture medium and the incubation time). Nevertheless, the extent of the modifications produced by these factors or conditions remains to be adequately described.

In this work, the evolution of some key physicochemical parameters of carbide ENM dispersions was studied using an *in vitro* biological assessment. A model ENM, TiC, was prepared in dispersion, due to its interesting mechanical properties for commercial applications. Two cell lines (IHK and A549) were assessed at different incubation times. PIXE (Particle-Induced X-ray Emission) was used to quantify the ENM fractions (supernatant and sediment), and CLS (Centrifuge Liquid Sedimentation) was used to determine the particle size distribution. In order to elucidate the contribution of the cell lines to this evolution, the same series of measures were performed only on each culture media (without cell lines). The results will describe the extent of the evolution produced in these key parameters, and the correlation on the used techniques will be discussed. The validity of the current assumption that the given dose equals the effective dose will be discussed in light of the findings.

N°P3a-52

STABILITY OF SIC AND TIC NANOPARTICLES DURING *IN VITRO* ASSESSMENT

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The evaluation of the risks associated to nanoparticles on the human health is a subject of crucial importance nowadays due to the extensive use of nanoparticles. Nanoparticles need to be “conditioned” to be adequately assessed. They need to be pre-dispersed in aqueous solutions and mixed in culture media, specific to the cell line, for *in vitro* assays.

In this work, several techniques (SEM, TEM, CLS, EDX, XPS) were used to adequately characterize the changes produced in the pre-dispersion of Silicon Carbide and Titanium Carbide nanoparticles. These two carbides were chosen in order to elucidate if a standard approach, a generic one, is valid for the evaluation of their potential toxicity. The results obtained from the pre-dispersion and the culture medium dispersion stages will be presented.

The results describe the modifications of some key parameters: the particle size distribution, the agglomeration state and the surface composition. The validity of the individual approach will be restated in light of the particle dependant modifications undergone from the raw conditions, the effective dose uptake and their dependence on the culture medium used.

N°P3a-53

CYTOTOXICITY OF MWCNT AND SiO₂ NANOPARTICLES AT DIFFERENT STAGES OF THEIR LIFE CYCLE AS NANOCOMPOSITE FILLERS

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Nanomaterials (NM) provide new features to already existing materials, and their production has increased exponentially since 1980s. MWCNT have already been used to produce nanocomposites (NC) with improved mechanical, thermal and electrical properties. SiO₂ NP are used in nanomedicine, to improve lithium-ion batteries or as reinforcers and flame retardants. Despite of the considerable knowledge generated during the last years on NM cytotoxicity, little is known on how these NM and their hazard change along their life cycle. Our goal was to evaluate the cytotoxicity of three nanomaterials at different stages of their life cycle as polymeric nanofillers. Polyamide-6 (PA6), one of the thermoplastic polymers most widely used in the industry, was used to generate the NC by extrusion and injection with three NM: MWCNT, hydrophilic SiO₂ and hydrophobic SiO₂. NM were extracted from NC by chemical dissolution of the polymer followed by filtration or centrifugation, and by calcination. The physical-chemical properties and cytotoxicity were evaluated for each NM at their raw stage and after extraction from the polymeric matrix before and after an accelerated aging process. In addition, raw NM and PA6 without additives were calcinated, as a control of the possible changes induced by this extraction process. Alveolar adenocarcinoma A549 cells and liver hepatocellular HepG2 cells were used to evaluate cell toxicity by the Alamar Blue® test.

The only method that proved to be valid for MWCNT extraction was calcination. Raw MWCNT, calcinated raw MWCNT, and MWCNT extracted by calcination of NC and aged NC, were tested in the cytotoxicity assays. Calcinated MWCNT were cytotoxic to the two cell lines evaluated (IC₅₀: 40 µg/mL). Moreover, MWCNT extracted from un-aged NC were cytotoxic to HepG2 cells (IC₅₀: 90 µg/mL). The rest of MWCNT evaluated and calcinated PA6 were not cytotoxic up to 100 µg/mL. Above this concentration, MWCNT interfered in the read-out of the viability tests.

Both filtration (after chemical dissolution) and calcination were the methods used to extract hydrophilic SiO₂. Hydrophilic raw SiO₂ was cytotoxic to both cell lines (IC₅₀: 459 and 139 µg/mL in A549 and HepG2, respectively). Hydrophilic SiO₂ extracted by filtration was slightly less cytotoxic (40% of inhibition at 500 µg/mL for A549; IC₅₀: 321.4 µg/mL for HepG2). Calcination markedly reduced its cytotoxicity (viability inhibited by 25% and 42% at 500 µg/mL in A549 and HepG2, respectively), independently of aging.

Hydrophobic SiO₂ had to be pre-dispersed with PVP (polyvinylpyrrolidone) before dilution in culture media for cytotoxicity testing. The two extraction methods selected for this NM were chemical dissolution followed by centrifugation, and calcination. None of the hydrophobic SiO₂ evaluated were cytotoxic in our working conditions up to the highest concentration tested (133 µg/mL).

Overall, calcination caused modifications on the NM that resulted in changes in their cytotoxicity. Due to the alterations introduced by the extraction procedure, the possible effect of aging on NC could not be discerned. In contrast, the extrusion and injection procedures, as well as dissolution followed by filtration or centrifugation, and the aging process had little impact on the SiO₂ properties: hydrophilic SiO₂ remained cytotoxic whereas hydrophobic SiO₂ was not.

N°P3a-54

IRON OXIDE NANOPARTICLES SHOW NO TOXICITY IN TERMS OF COMETE ASSAY IN LYMPHOCYTES: A PROMISING VEHICLE FOR NITRIC OXIDE RELEASING NANOCARRIER IN BIOMEDICAL APPLICATIONS

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Superparamagnetic iron oxide nanoparticles (SPIONs) have been the subject of intensive investigations due to their important properties and biomedical applications, such as magnetic resonance imaging (MRI), and drug delivery. The surface of the nanoparticle must be coated with biocompatible hydrophilic materials, so that the coated particles can form a stable aqueous dispersion. Stable non-toxic aqueous dispersion of SPIONs are usually obtained by coating the magnetic nanoparticles with biocompatible and hydrophilic ligands. It was previously reported that uncoated SPIONs may have toxic effects on cells. However, the cytotoxicity of coated nanoparticles is rather influenced by the outer coating layer covering the SPIONs than by the iron oxide nanoparticles themselves. In this work, SPIONs were coated with two thiol-containing hydrophilic ligands: mercaptosuccinic acid (MSA) and dimercaptosuccinic acid (DMSA). Genotoxicity of DMSA- and MSA-coated SPIONs was evaluated in human lymphocyte cell by comete assay. Figure 1 shows the DNA damage of the cells after incubation for 1 h with DMSA- and MSA-coated SPIONs, as well as free DMSA and free MSA.

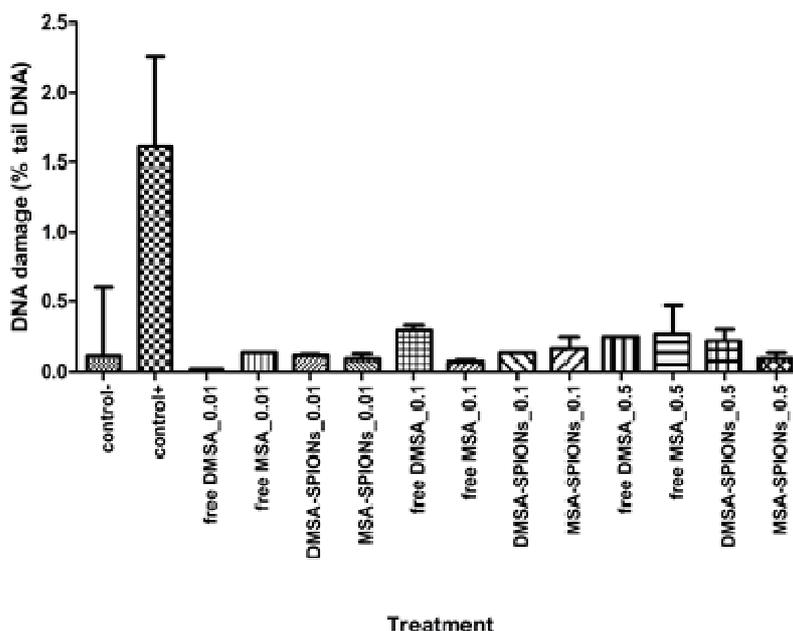


Figure 1. DNA damage (% tail DNA) of human lymphocyte cells after incubation for 1 h with DMSA- and MSA-coated SPIONs, as well as free DMSA and free MSA at concentrations (0.01, 0.1, and 0.05 mg/mL, as indicated in the Figure).

This result shows that SPIONs coated with DMSA and MSA are not genotoxic, at high concentrations. As a consequence, SPIONs capped with thiols are an interesting option for biomedical applications in drug delivery. Moreover, this nontoxic and biocompatible surface coating has an important role for drug delivery: it is a site for a chemical modification with a biological important molecule to allow a targetable delivery. In fact, our preliminary data indicated the these nanoparticles can be used to carry and delivery nitric oxide (NO), by the nitrosation of thiols groups present on the nanoparticles surface. NO-releasing SPIONs show an increase in genotoxicity at higher concentrations. Current studies are in progress based on the genotoxicity of NO-releasing SPIONs. This material may be used to delivery NO directly to target tumor cells, where NO has anti-tumor activities.

N°P3a-55

CYTOTOXICITY AND GENOTOXICITY OF BIOGENIC SILVER NANOPARTICLES

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The important antimicrobial activities of silver nanoparticles induced a wide application in several sectors as medical, agricultural and food industries. At the same time a concern to the biological impact due to a large production of these materials and the possible risks to the environment and health are actually of the priority importance. Then, it is necessary to study the capacity to increase damage to the genetic material, since nanoparticles are able to cross cell membranes and reach the cellular nucleus. In this direction our interest is to study the potential nanoparticle genotoxicity, including the effects of different nanoparticle sizes and methods of synthesis since little is known about the genotoxicity of different silver nanoparticles and their effects on the DNA of organisms. Biogenic nanoparticles were prepared by *Fusarium oxysporum* in ~0--- nm and zeta potential of - --- mV.

Pry precisamos os dados de tamanho e potencial zeta e outros dados da nanoparticulas.

Renata: aqui devemos colocar os resultados em texto. Podemos colocar uma tabela ou uma figura ao final e sem referencias. Ao final tem um modelo do congresso.

This present work concludes that biogenic silver nanoparticles are generally less cyto/genotoxic in vivo compared with chemically synthesized nanoparticles. Furthermore, human cells were found to have a greater resistance to the toxic effects of silver nanoparticles in comparison with other organisms.(esta conclusão pode ser trocada)

N°P3a-56

BIOCOMPATIBILITY AND CYTOTOXICITY STUDY OF NANOPHOTONIC CONTACT LENS MATERIAL

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Due to more often application of biomaterials and nanomaterials, the studies of implanted materials and tissue interactions have become very important. Biocompatibility is the ability of a material to express appropriate response upon specific application that is to be non-toxic and not damaging to biological systems. Since materials in nano state have different characteristics and due to the size of particles their penetration into tissues is easier, their biocompatibility should be precisely and specifically investigated. Fullerenes are the third carbon allotrope forming the class of spherical three-dimensional molecules. The least stable and at the same time the most common in terms of natural occurrence is fullerene C₆₀, also known as the *Buckyball*. The combination of unusual characteristics makes fullerenes very attractive family of molecules for the wide range of biomedical applications. One of the main disadvantages in fullerene applications is its low solubility in water. In order to make them soluble, they must be functionalized with polar groups such as –OH and –COOH. From all the water soluble fullerenes the most important ones are those with –OH groups attached, named fullerols or fullerlenols. We have added fullerene (C₆₀) and fullerol (C₆₀(OH)₂₄) into PMMA and developed new materials for contact lenses.

In this paper we present the investigation of influence of new nanophotonic materials for contact lenses on aqueous solutions which are similar to tear film. The aim of the investigation was to compare the influences of those materials on different solutions, such as aqua purificata, saline and drops for dry eyes. For the analysis of different solutions, we used optomagnetic and IR spectroscopy. The acquired spectrums were commented and compared with the standard contact lens material, which was analyzed by the same method, in order to show the differences in influence of this standard and new nanophotonic material. Also the cytotoxicity test on extract of nanophotonic contact lenses with incorporated C₆₀ was conducted to evaluate the potential for cytotoxicity. This study was conducted according to the requirements of the United States Pharmacopeia and ISO 10993 standard: Biological Evaluation of Medical Devices, Part 5 (2009): Tests for in vitro cytotoxicity. In general this research contributes to better understanding of the biocompatibility of new contact lens materials.

N°P3a-57

NANOPARTICLES IN PAINTS; A NEW STRATEGY TO PROTECT FAÇADES AND SURFACES?

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Painted façades are easily colonized by microorganisms (algae, bacteria, fungi, lichens, etc.), if they don't contain a biocidal protection. The colonialization by microorganisms is an aesthetic problem. However microorganisms are also actively involved in the weathering and deterioration of façades and paints. Highly ecotoxic, as well as non-degradable biocides with a wide protection spectrum have been abolished by law (Biocidal Products Directive 98/8/EC). The paint and lacquer industries are therefore considering using nanosilver, photocatalytic active nanotitanium dioxide and nanosilica dioxide as additives for the protection of paints, against microbial, physical and chemical deterioration. At the time it's not clear, if nanoparticles in paints and façades will achieve the proposed effects, since there are no long time studies available. Another fact is that the potential risks of nanoparticles for the environment and the human health is controversial discussed.

The most sensitive entry port for nanomaterials is the lung. However other organs / systems may also be affected by nanoparticles. Therefore the aim of the study was to assess the potential hazard effects of the three most interesting particles for paints on the immune system and the gastro-intestinal tract in vitro.

The physical-chemical properties of these commercial available particles were characterized and the existence of potential endotoxin contaminations was determined. Release of reactive oxygen species (ROS), inflammation, live/death staining and for subtoxic concentrations the potential genotoxicity were assessed. Nanoparticle-uptake and cell morphology were further investigated.

In our studies with cells from the gastro-intestinal tract (CaCo-2) and immune cells (T-lymphocytes, Jurkat) could be demonstrated that nanosilver (TEM-size 25 nm to 90 nm) was far less toxic than silver ions of comparable concentrations. A significant amount of necrotic cells could be observed after exposure of CaCo-2 cells to 27 µg/ml nanosilver for 2 days. The effect on cell death was much more severe, when silver was applied in ionic form (as silver sulphide). An exposure to 1 µg/ml ionic silver for 2 days caused a very high amount of necrotic cells.

Nanotitanium dioxide can adsorb UV-light and in the presence of water, hydroxyl radicals are generated photocatalytically. These hydroxyl radicals are strong oxidizers and are able to kill growing microorganisms. The exposure of CaCo-2 cells and Jurkat cells to photocatalytically active nanotitanium dioxide (Hombikat UV 100, TEM-size 15 nm) under dark conditions, didn't affected the cells significantly. However, the cells were able to incorporate nanotitanium dioxide, especially when cells were exposed to higher concentrations. Nanosilica is improving the properties of the paints by increasing the water repellence. When cells were exposed to 243 µg/ml nanosilica dioxide (TEM-size 19 nm) for up to 2 days no cytotoxic effect could be observed.

N°P3a-58

PULMONARY TOXICITY AFTER INHALATION OF SILICON CARBIDE NANOPARTICLES IN RAT.

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Silicon carbide nanoparticles (SiC) are used for many applications as in lithium-ion batteries, in cosmetics, and in particular in ceramic plates of bulletproof jacket vests. Due to their increased presence in daily life, it appears important to evaluate the impact of SiC on the pulmonary tract. We evaluate them after acute inhalation (6 hours per days) in a whole body exposure rat model. Rats are sacrificed directly after the 6 hours of exposure, 24 hours or 72 hours later. Many analyses are performed in bronchoalveolar lavage fluid (BALF) as cells count and differentiation, lactate dehydrogenase and total proteins dosage. A complete histopathological examination is also performed. Analyses shown that SiC can induce lung inflammation after inhalation with the presence of neutrophils and eosinophils in the BALF. The macrophages observation indicates that they are filled with SiC. We can conclude that SiC are able to induce lung inflammation after inhalation in rat.

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N°P3a-59

DEVELOPMENT AND VALIDATION OF A WHOLE-BODY INHALATION EXPOSURE MODEL FOR THE EXPOSITION OF RATS TO NANOPARTICLES AEROSOL

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* Both authors have participated equally in the study reported here.

The pulmonary tract is the main exposure pathways to nanoparticles (NPs). In fact, humans are exposed every day to diesel exhaust particles containing NPs. In manufacturing industries, workers can be accidentally exposed to NPs due to the defect of ventilation system or individual protective equipment. The physicochemical property of NPs most considered for pulmonary tract exposure is their size. The size and shape of a particle determines aerodynamic properties that govern entry, depth of penetration, and deposition in the lung. The aim of this study is to evaluate the pulmonary impact of NPs after inhalation in a rat model. In fact, inhalation model is closer to the reality than instillation for reproduction of working conditions, dose generated, and profile of deposition in the pulmonary system. Inhalation is also more complex to implement and requires expansive equipments as aerosol generator and analyzer. We develop and validate a whole-body inhalation exposure model for rats in which animals are exposed to an aerosol of NPs. It appears also important to have a continuous monitoring of the NP concentration in atmosphere during the experiment. Silicon carbide (SiC) are selected for the model development and the pulmonary toxicity study.

Keywords

SiC nanoparticle, lung, whole-body, toxicity

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N°P3a-60

**FUNCTIONALIZED DOUBLE WALLED CARBON NANOTUBES (DWCNTS) FOR TARGETED
DRUG RELEASE**

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Nanotechnology is a most promising field for generating new applications in medicine. Carbon nanotubes (CNT) exhibit many unique intrinsic physical and chemical properties and have been intensively explored for biological and biomedical applications in the past few years. Their unique surface area, stiffness, strength and resilience have led to much excitement in the field of pharmacy. Carbon Nanotubes holds good for desired drug delivery systems for the treatment of cancer, gene transfer and DNA applications. We have synthesised highly graphitised double walled carbon nanotubes (DWCNTs) using FeMoMgO catalyst by simple chemical vapour deposition (CVD) techniques at low temperature. Double-walled carbon nanotubes (DWCNTs) were selectively functionalised by treatment with concentrated nitric and sulphuric acid, resulting in carboxylated and characterized by various physicochemical techniques like x-ray diffraction (XRD), scanning electron microscope (SEM), transmission electron microscope (TEM) and Raman spectroscopy. Functionalized carbon nanotubes (f-CNTs) are emerging as new tools in the field of nanobiotechnology and nanomedicine.

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N°P3a-61

BIODISTRIBUTION STUDIES ON NANOPARTICLES ARE SO FAR OF LIMITED USE FOR PBPK MODELING

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The health hazards with nanoparticles (NP) are largely unknown, and human data are unlikely to be generated to any great extent. Previous experience with industrial chemicals and pharmaceutical drugs shows that combined use of animal uptake and biodistribution studies and physiologically-based pharmacokinetic (PBPK) modeling is useful in human health risk assessment and this approach should be useful also for NPs. However, it requires that animal experiments are carried out and reported in an appropriate way. The aim of this study was to review all published data on the biodistribution of intravenously injected NPs. By this approach the additional complexity of absorption is avoided. Data were mainly retrieved for gold, silver, titanium dioxide, silica and polymeric NP. Very few of the 66 reviewed articles, covering 244 different NPs, seem useful for PBPK modeling. The following major limitations were identified: (1) incomplete NP and dose characterization, (2) short follow-up post-dosing, (3) few samples per tissue, (4) few tissues/organs studied, and (5) failure to account for the mass balance in tissues, and (6) lack of confirmation on integrity of nanoparticle in tissue. These shortcomings make time course descriptions, half time calculations, estimates of bioaccumulation uncertain. Most studies present data for blood, liver and spleen, many also for lungs and kidneys. A few studies suggest that NP deposits in muscle, bone and carcass should not be neglected. Overall, our review indicates that it is difficult to draw general conclusions about the biodistribution of NPs. With the limited data at hand, it seems that no individual factor such as size, coating, shape, charge, chemical composition or agglomerations status can explain the biodistribution. In conclusion, the biodistribution of NPs is complex and additional studies are needed. To be useful in PBPK modeling, these studies should include more complete NP characterization, cover many organs and time points, have long follow-up, and account for the mass balance. It would be valuable to develop a standard protocol for uptake and biodistribution studies of NPs. This study was financed by a grant from the Swedish Council for Working Life and Social Research.

N°P4a-1

EVALUATION OF TOXICITY OF THE NANOSCALE MATERIALS FOR MAMMALIANS AND ENVIRONMENT

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Introduction. Rapidly developing production and growing scales of application of nanocomposites in different fields of industry, agriculture, medicine, and common human life also predict potential dangers of these materials for human and environment. Unfortunately, there are no generally accepted risk assessment methods for evaluation of toxicity of the nanoscale materials for human and environment.

Two aims were addressed in this study: 1) to compare *in vitro* and *in vivo* action of highly toxic anticancer drug doxorubicin when delivered in free form or being coated with the developed bio-functionalized nanoscale polymeric shell; 2) to compare the action of the bio-toxic element Co²⁺ targeting fresh water mollusk and fish as a chloride salt or coated with nanoscale polymeric composite; develop sensitive biochemical approaches for assessing bio-risks associated with using Cobalt-containing nanocomposite.

Synthesis of novel nanoscale polymeric olygoelectrolytes was performed via radical copolymerization of vinyl pyrrolidone (NVP), 5-(*tert*butylperoxy)-5-methyl-1-hexene-3-yne (VEP) and dimethyl aminoethyl methacrylate (DMAEM) (molar ration = 1:1:1) in the dimethyl formamide solution. Synthesis of coordinated Cobalt-containing nanocomposite was carried out in the reaction of Co²⁺ with similar (as noted above) polymeric substances in ethanol.

To address the 1st aim, the developed polymeric nanocomposites were used for delivery of anticancer drug Doxorubicin. It was found that such encapsulation makes possible achieving similar treatment effect both *in vitro* (mammalian tumor cells) and *in vivo* (tumor-bearing mice) even when this drug was used in 10 times less doses. The encapsulated drug was much faster up-taken by the targeted cells, accumulated in their nucleus, and its cytotoxic effects were accompanied by typical pro-apoptotic changes. No visible negative side effects were found in the treated tumor-bearing mice.

To address the 2nd aim, we studied the modulation of toxicity of Co²⁺ towards freshwater bivalve mollusc *Anodonta cygnea* and fish *Carassius auratus*. That was done by applying this element as a complex with polymeric nanocomposite. These organisms were subjected for 14 days to the action of Cobalt-nanocomposite, or the corresponding concentrations of Co²⁺ (50 µg/L), or the polymeric nanocomposite. Cobalt concentration was ecologically relevant, and corresponded to its concentrations in blood of persons with worn implants. The multi-marker approach was used to evaluating the bio-risks associated with applying novel metal-containing polymeric nanocomposites. Principal value of a novel biomarker - metal-buffering stress-proteins metallothioneins - for early warning of bio-toxic effects of some nanocomposites in surface fresh waters was experimentally grounded. Co²⁺ demonstrated marked toxicity in the mollusks at tissue and biochemical levels, while its complex with the polymeric nanocomposite was much less harmful there. In fish (*C. auratus*), Cobalt-containing polymeric nanocomposite was more harmful than Cobalt used as a salt.

Conclusions. Encapsulation of anticancer drug Doxorubicin in specific polymeric nanocomposites enhances its delivery into targeted mammalian cells and its cytotoxic action, as well as protects against negative side effects in the organism. Different ways of decreasing harmful effects of the nanocomposites towards mammals (human) and environment (freshwater mollusk and fish) are considered.

N°P4a-2

**METAL OXIDE NANOPARTICLE TRANSPORT IN POROUS MEDIA – AN ANALYSIS ABOUT
(UN)CERTAINTIES IN ENVIRONMENTAL RESEARCH**

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“There is a high level of uncertainty in the research concerning the fate and behavior of nanoparticles in the environment” is often stated. Research about the fate and behavior of engineered nanoparticles in the environment is, despite its wide applications, still in the early stages. The related scientific uncertainty is partly due to the lack of a methodology for separation, detection, and characterization of nanoparticles in complex, environmental matrices. One way nanoparticles may enter the environment is via sewage plants to the aquatic and terrestrial systems. Soils can play a key role for the fate and behavior of nanoparticles, because soils can act as a nanoparticle-sink as well as a nanoparticle-source, e.g. for groundwater, organisms, and plants. One key parameters of the fate of nanoparticles in the environment is their mobility.

The objective of this study is to structure the certain knowledge, to analyze the known uncertainties and to characterize the identified knowledge gaps concerning mobility of engineered metal oxide nanoparticles in porous media. In this presentation, the current state of the art of the environmental fate of engineered nanoparticles is presented and discussed with respect of the certain knowledge. Therefore, from the current literature, selected key papers were analyzed concerning to the way how scientific certainty was addressed and how scientific uncertainty was considered and discussed related to mobility of metal oxide nanoparticles in terrestrial environments.

The mobility of nanoparticles is mainly investigated in model laboratory studies under well-defined conditions, which are often not realistic for natural systems. In these model systems, nanoparticles often retain in the pore system due to aggregation and sedimentation. However, under environmental conditions, the presence of natural organic matter may cause stabilization or disaggregation of nanoparticles and favors therefore higher mobility of nanoparticles. Additionally, potential higher mobility of particles using preferential flow paths is not considered. Knowledge of the long-term behavior of nanoparticles concerning disaggregation, dissolution or remobilization in soils under environmental conditions is scarce. Moreover, the state of nanoparticle coatings after released from products or after a certain time being under environmental conditions is unknown.

Scientific uncertainty itself is rarely mentioned in the research papers. Seldom known methodically uncertainties in nanoparticle characterization are referred to. Due to the sparse studies concerning natural material or natural pore systems, certain conclusions concerning the mobility of nanoparticles in the soil environment are not possible to draw.

N°P4a-3

ASSESSING THE HETEROAGGREGATION OF MANUFACTURED NANOPARTICLES WITH NATURALLY OCCURRING COLLOIDS IN A TYPICAL SURFACE WATER

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To study and predict the fate of engineered nanoparticles (ENP) in surface water, relevant environmental conditions should be applied, regarding both the system composition and the ENP concentration. These factors mainly determine together the fate scenario of ENPs, potentially interacting with the surrounding components of the system. Indeed, under typical surface water conditions, the concentration of ENP falls in the microgram per liter range, while natural mineral colloids and organic matter are very abundant in suspension. This is likely to favor significantly the heteroaggregation mechanisms rather than the commonly studied homoaggregation of the ENPs.

In this work, we studied the interactions occurring between ENPs and natural colloids as a function of the physicochemical conditions of the solution and of the ENP concentration in a relevant range (< 1 mg/L). Pure TiO₂ nanoparticles of 20 nm size were studied, while pure montmorillonite clay and silica with 0.5 - 1 μm size were used as colloid surrogates at 100 mg/L in a pure water of controlled pH, ionic strength and natural organic matter. Adsorption isotherms were realized to quantify the affinity of the colloids for the ENPs. Laser diffraction was used to determine the heteroaggregation kinetics of the colloids by the ENPs as a function of the abovementioned parameters.

It appears that, depending on pH and ionic strength, ENPs show a significant affinity for the colloids, which induces rapid heteroaggregation of the system and sedimentation of the aggregates formed. This phenomenon is comforted by the fact that both colloids and ENPs remain well dispersed in suspension when introduced separately into the same solution. Moreover, the measured heteroaggregation rate is higher than the highest rate measured elsewhere in salt-induced rapid homoaggregation of the colloids. In the example shown in the figure below, the effect of the concentration ratio between ENP and colloid appears highly determining for the heteroaggregation mechanism. This indeed relates to the key number of absorbed ENPs that assume bridges between neighbor colloids.

These data, coupled to a fate model, will enable to deliver a probability ranking of the potential scenarios on the fate of ENPs in natural aqueous systems at the river scale.

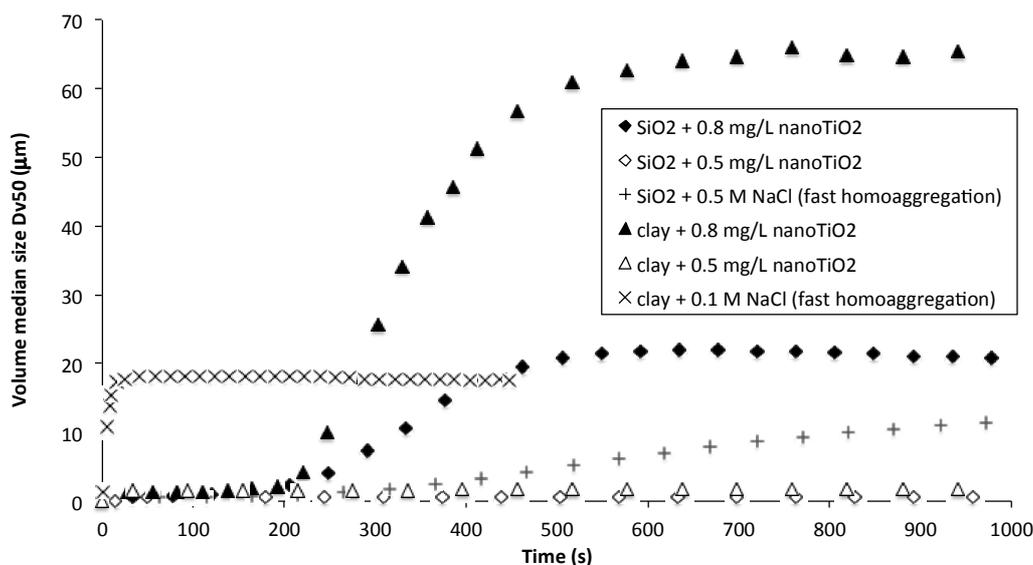


Figure: Aggregation kinetics of colloids by nanoparticles (heteroaggregation) or salt (homoaggregation) measured by laser diffraction – Time-resolved evolution of the median aggregate size as a function of colloid type and nanoparticle concentration introduced at time 0. Colloid concentration = 100 mg/L ; pH = 5; I = 0.001 M NaCl.

N°P4a-4

POTENTIAL SAFETY IMPLICATIONS OF NANOFORMULATION OF AGROCHEMICALS IN CROPS PRODUCTION

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Traditional agrochemicals, mainly including two categories, chemical fertilizers and pesticides, are playing extremely important roles in enhancing crop productivity and preventing from biological disasters. The annual input amounts of fertilizers and pesticides have reached respectively to 170 million tons and 3.5 million tons worldwide. More than 70% of fertilizers and 90% of pesticides run off into the environment and residue in agricultural products in process of application. Inefficient use of agrochemicals causes a series of ecological environment problems, such as non-point pollution, water body eutrophication, soil degradation, and loss of biodiversity. Recent years, using nanotechnology to formulate nano-delivery systems (or nanoformulations) for pesticides and nutrients by virtue of nanomaterials related properties has shown a great potential for alleviation of these problems. Application of nano-delivery systems for pesticides focuses on enhancing efficacy and reducing spray drift, while fertilizers focuses on problems of bioavailability due to soil chelation, over-application and run-offs. The development of agrochemical nanoformulation aims at precise release of necessary and sufficient amounts of their active ingredients over a period of time, in responding to environmental triggers and biological demands through targeted delivery or controlled release mechanisms. However, these advantages might be offset by some potential risks of human health and ecological disasters, caused by the nanoparticles flowing into the environmental systems and food chains. Nanoparticles in agrochemicals may involve either very small particles of insoluble or fat-dispersible active ingredients and nanocarrier materials. The environmental Toxicology studies of these nanoparticles should be conducted in following aspects.

1. The pathway of agricultural nanoparticles flowing into the environment

The pathway and volume of nanoparticles from agrochemicals flowing into environment in the process of leaf spray and soil application.

2. Environmental behaviors of agricultural nanoparticles

(1)The process of transportation and transformation of agricultural nanoparticles in environmental system and the food chain, such as dispersion, congregation, absorption, bio-uptake, bioaccumulation, metabolism and degradation.

(2)The main factors affecting transport and transformation of agricultural nanoparticles, such as particle size, adsorption capacity, aggregation and dissociation degree, natural degradation capacity, soil properties and water body characteristics.

(3)The micro interface behavior of agricultural nanoparticles in leaf surface, roots and soil environment.

(4)Changes in physico-chemical properties and toxic effects after agricultural nanoparticles flowing into the environment and life system.

(5)Persistence, recyclability and overall sustainability of agricultural nanoparticles in environmental system.

3. Toxicity of agricultural nanoparticles to environment life

Toxicity of agricultural nanoparticles to environment life, such as bees, birds, fish, silkworms, earthworms, soil beneficial microorganisms. These toxicity evaluation include acute toxicity, chronic toxicity, cumulative toxicity and joint toxicity, in vivo stability, bioaccumulation and biomagnification, etc.

4. Toxicological effects of agricultural nanoparticles on plants

The adverse effects of agricultural nanoparticles on physiological processes, stress resistance, growth and development state, crop yield and quality.

5. Agricultural product residues and food safety

The distribution and behavior characteristics of agricultural nanoparticles inside of crop plant, such as absorption, transport, accumulation, degradation, etc, especially the transportation and accumulation towards the edible organs and metabolism increasing toxic phenomenon.

N°P4b-1

ENVIRONMENTAL DISSEMINATION OF SILVER NANOPARTICLES: WHICH IMPACT ON CROPS?

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With the increasing use of nanobased-products, nanoparticles (NPs) are likely to be disseminated in the environment (soil, water, air). Crops may be exposed to these NPs through their root system or through their aerial parts due to deposition of atmospheric particles. NPs may be sorbed on the root or leaf surface, or may be transferred inside the plant tissues. The extent of these processes, the mechanisms involved in NP transfer into crops and the fate of NPs inside plants are poorly understood.

In this study, lettuce plants (*Lactuca sativa*) were exposed to a foliar contamination of Ag NPs coming from paints by application of a suspension of NPs (nominal diameter 66 nm, 1 µg Ag/mm² foliar surface). After one week, the Ag content in the leaves after washing with water was measured in the perspective of human exposure by consumption of crops. The impact in terms of phytotoxicity was assessed by measuring the biomass, chlorophyll content and oxidative stress biomarkers. The distribution and the speciation of Ag in the leaves were studied by SEM- and TEM-EDX, synchrotron based micro X-ray fluorescence (µXRF) and micro X-ray absorption spectroscopy (µXAS) on cryosections, and time of flight secondary ion mass spectrometry (ToF-SIMS).

Washed lettuce leaves contained 17.3 mg Ag. kg⁻¹ fresh weight. The biomass and chlorophyll content were not affected by Ag exposure. Imaging techniques showed the presence of both large (few µm) and relatively small (<200 nm) agglomerates of Ag on the leaf surface. Both types of agglomerates were also observed inside leaves, and large Ag accumulations were frequently observed in the sub-stomatal cavity (Figure 1) and as smaller or more diffuse regions. Ag was found in the epidermis, in the parenchyma and in vascular tissues. Diffuse Ag concentrations around Ag-rich spots suggested some dissolution processes. µXAS analyses confirmed the oxidation of elemental Ag and the formation of Ag⁺ organic complexes as secondary species.

These results suggest that crops exposed to atmospheric deposition of NPs may undergo contamination due to foliar transfer of NPs.

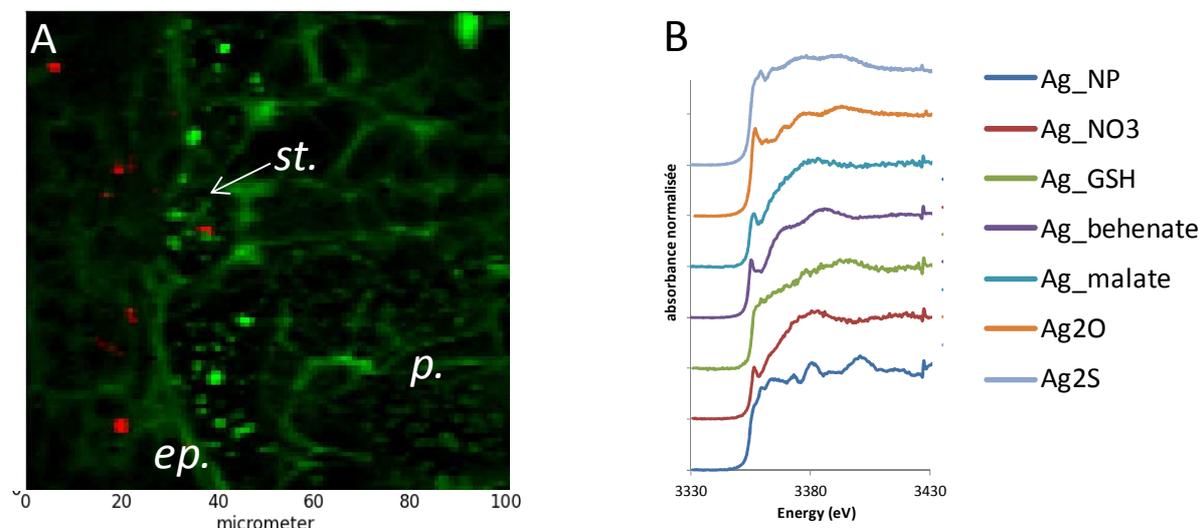


Figure 5: A. Ag (red) and K (green) distribution in lettuce leaves after foliar exposure. (ep. epidermis, p. parenchyma, st. stomata). B. Ag reference compounds analyzed by µXAS.

N°P4b-2

**BIO-INTERACTIONS BETWEEN PROTEINS AND CeO₂-NPS
WITH SIMILAR HYDRODYNAMIC RADIUS**

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Our study project focuses on bio-interactions between proteins and CeO₂ nanoparticles (CeO₂-NPs). Currently, the toxicity of CeO₂-NPs, the use of which is increasing in medical applications, cosmetic products, polishing materials and by products from automotive fuel additives (Park *et al.*, 2008), has been demonstrated in several studies on different prokaryotic or eukaryotic cell lines (Auffan *et al.*, 2009).

CeO₂-NPs used in this study were synthesized by aqueous precipitation of Ce⁴⁺ (NO₃)₄ salt at acidic pH. Transmission electron microscopy and X-ray diffraction measurements show ellipsoidal crystallites of cerianite with a mean diameter of 8 nm and a specific surface area of 400 m²/g (Thill *et al.*, 2006).

The mean hydrodynamic radius of these CeO₂-NPs is 8 nm and this size is similar or greater than that of most proteins. The "protein corona" concept developed by Dawson *et al.* (Lynch *et al.*, 2007) implies that the size of the adsorbed molecules is much smaller than the NPs, and thus in our case the question is raised of how NPs and proteins interact. Indeed, understanding the mechanisms of CeO₂-NPs-protein interaction is essential to explain their biological and potential toxicological effects. Correct molecular conformation is essential for proteins to work as intended and slight conformational changes can disturb the protein's function. (Elsaesser *et al.*, 2011)

We have studied the CeO₂-NPs interacting with proteins of different sizes through Dynamic Light Scattering (DLS) and Circular Dichroism studies to understand if the interactions involve specific amino acids or not. The results have been analyzed, taking into account the aggregation states of the CeO₂-NPs by using DLS. The conformational change of proteins after their interaction with CeO₂-NPs and the involvement of specific sites will be presented. The "protein corona" concept in this specific case will be discussed in the light of the results obtained for CeO₂-NPs.

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N°P4b-3

CARBON NANOTUBES ENHANCED THE LEAD TOXICITY ON THE FRESHWATER FISH

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Carbon nanotubes are promising nanostructures for many applications in materials industry and biotechnology. However, it is mandatory to evaluate their toxicity and environmental implications. In this work, we have evaluated the nitric acid treated multiwalled carbon nanotubes (HNO₃-MWCNT) toxicity on the freshwater fish, Nile Tilapias (*Oreochromis niloticus*) as well as the modulation of lead (Pb) toxicity after the nanotube interaction. Firstly, industrial grade multiwalled carbon nanotubes [Ctube 100, CNT Co. Ltd] were treated with 9M HNO₃ for 12h at 150°C aiming to generate oxygenated groups on the nanotube surface that improve their water suspension and heavy metal interaction. The HNO₃-treated multiwalled carbon nanotubes were physico-chemically characterized by using several techniques [e.g. TEM, FE-SEM, TGA, ζ-potential, UV-visible and Raman spectroscopy]. The Nile Tilapias juveniles (n=240) were exposed by using the static renewal method at pH 7.2 and 20 °C. The HNO₃-MWCNT sample has not demonstrated toxicity on Nile Tilapias from 0.1 to 3.0 mg/L until 96h. The 24, 48, 72 and 96h LC₅₀ value of Pb were 1.65, 1.32, 1.10 and 0.99 mg/L, respectively. Aiming to evaluate the influence of Pb-nanotube interaction on the ecotoxicity, we have submitted the Nile Tilapias to different concentrations of Pb mixed with a non-toxic concentration of HNO₃-MWCNT (0.5 mg/L). The 24, 48, 72, 96 h LC₅₀ values of Pb plus nanotubes were: 0.32, 0.25, 0.20, 0.18 mg/L, respectively. These values showed that there is a synergic effect after Pb-nanotube interaction because it was observed an increase over five times of Pb toxicity on the freshwater fish (**Figure 1**). In this sense, X-ray energy dispersive spectroscopy (EDS) was used to confirm the adsorption of lead on the carbon nanotube oxidized surface. Furthermore, the exposure of HNO₃-MWCNT on the Nile Tilapias caused a decrease of oxygen consumption and ammonium excretion, relative to the control. Finally, our results draw attention to the environmental implications of the carbon nanotubes and their interaction with classical pollutants.

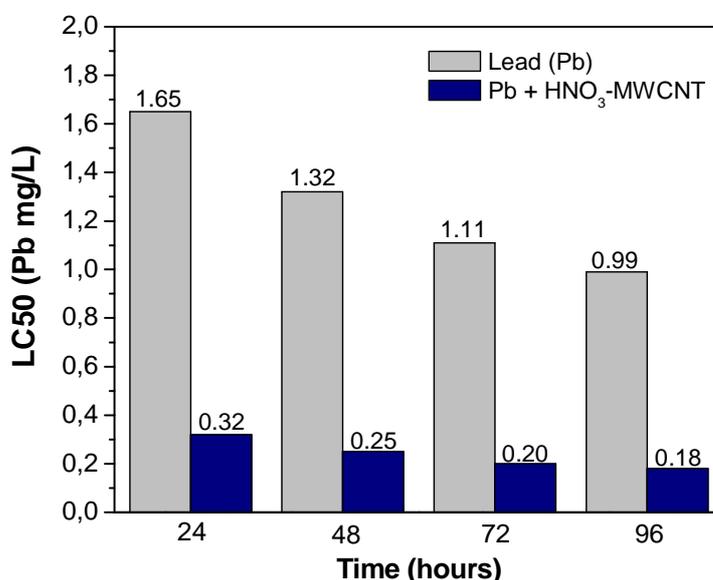


Figure 1. Modulation of the LC₅₀ toxicity value of lead (Pb) on the freshwater fish, Nile Tilapias (*Oreochromis niloticus*) after the interaction with nitric acid treated multiwalled carbon nanotubes (HNO₃-MWCNT). It was used 0.5 mg/L of the nanotubes during this experiment, a non lethal dose on the freshwater fish.

N°P4b-4

COLLOIDAL STABILITY OF MODIFIED CARBON NANOTUBES

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The implications of a carbon nanotube (CNT) release into the environment are majorly influenced by the colloidal stability of the CNTs in surface or ground waters. A main factor for this stability is the degree of oxidation of the CNTs. Pristine CNTs show very low dispersion stability in water and quickly aggregate and sediment. However, oxidation processes are expected to happen in the environment or are deliberately performed on the CNTs, for better dispersability in technical applications prior to the possible release.

For this study samples of different types of CNTs, multi wall as well as single wall CNTs, were oxidized by a microwave assisted acid treatment with concentrated nitric and sulphuric acid, which allowed a fast reproducible modification of the CNTs. The modification was verified by IR and Raman spectroscopy, thermogravimetric analysis and measurements of the iso-electric point.

The colloidal stability of the so modified CNTs was investigated using dynamic light scattering methods to follow the aggregation and sedimentation of the CNTs as well as establishing the zeta potential of the modified CNTs in waters of different ionic strength.

The colloidal stability of the CNTs in distilled and low ionic strength water exceeds months. This has implications for their transport behaviour upon release into the environment. A transport of CNTs in surface and ground waters can be expected under certain conditions.

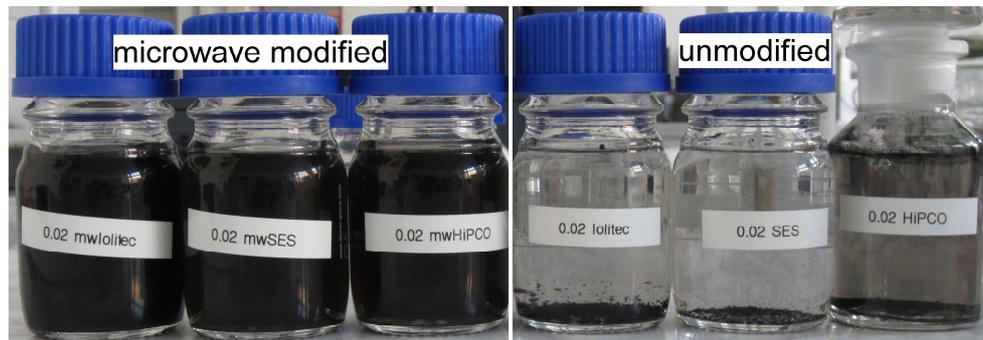


Fig. 1: Picture of different modified and unmodified CNT samples in distilled water after 1 day of sedimentation time.

N°P4c-1

**BEHAVIOURAL RESPONSES OF *HEDISTE DIVERSICOLOR* (POLYCHETA)
TO Ag, CdS, OR CuO NANOPARTICLES USING THE MULTISPECIES FRESHWATER
BIOMONITOR® (MFB)**

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Engineered metal nanoparticles are extensively used in diverse applications. Because aquatic environments are the ultimate “sink” for all contaminants, it is expected that nanoparticles (NP) will follow the same fate. Despite a lot of work on toxicity of nanoparticles on seawater and freshwater species, there is a little number of studies dealing with sediment (Pang et al., 2012). Biochemical biomarkers have been successfully used to unveil potential stress due to engineered nanoparticles (Buffet et al., 2011). Behavioural biomarkers have been proposed as ecologically relevant biomarkers since they are indicative of responses at the individual level that can be connected to effects at the population level.

The aim of the present study is to investigate behavioural responses of AgO NPs, CdS NPs and CuO NPs on the endobenthic ragworm *Hediste diversicolor*, used as sentinel species in marine environment biomonitoring. Experiments were conducted under environmentally realistic conditions in outdoor mesocosms for AgO NPs and CuO NPs or under laboratory conditions for CdS NPs. Organisms were exposed, for two weeks, to 10 µg.L⁻¹ as AgO NPs, CdS NPs and CuO NPs or as soluble Ag, Cd and Cu. Multispecies Freshwater Biomonitor (MFB), based on quadropole impedance conversion, is a method that improves behavioural analysis by registering online movements of the organisms in test chambers when they are already burrowed.

Two different behavioural patterns have been characterized : i) burrowing with undulation occurring at 0.5 Hz frequency and ii) head movement, when feeding, occurring at 1 to 2 Hz frequencies. The average time spent in burrowing/undulation and head movement/feeding behaviours showed that no significant effect occurred after 7 and 14 days of exposure to both forms of Ag, whereas for Cd significant impaired activities started from the first week for ragworms exposed to both forms of Cd (CdS NPs and soluble Cd) and only from the 14th day of exposure to Cu either as CuO NPs and soluble Cu. These impairments of locomotion and feeding behaviour may have direct effects on the capability of animals to escape from predators, to capture preys, etc... and reinforce the suitability of behavioural biomarkers as very sensitive tools to assess the impact of the contaminants at concentrations far below the lethal effect.

This study is the first experiment using MFB for recording behaviour of *H. diversicolor* in a sensitive and quantitative way.

*Buffet, P.-E.; Tankoua, O. F.; Pan, J.-F.; Berhanu, D.; Herrenknecht, C.; Poirier, L.; Amiard-Triquet, C.; Amiard, J.-C.; Bérard, J.-B.; Risso, C.; Guibbolini, M.; Roméo, M.; Reip, P.; Valsami-Jones, E.; Mouneyrac, C., Behavioural and biochemical responses of two marine invertebrates *Scrobicularia plana* and *Hediste diversicolor* to copper oxide nanoparticles. Chemosphere 2011, 84, (1), 166-174.*

*Pang, C.; Selck, H.; Misra, S. K.; Berhanu, D.; Dybowska, A.; Valsami-Jones, E.; Forbes, V. E., Effects of sediment-associated copper to the deposit-feeding snail, *Potamopyrgus antipodarum*: A comparison of Cu added in aqueous form or as nano- and micro-CuO particles. Aquatic Toxicology 2012, 106–107, (0), 114-122.*

Acknowledgement - European Community's Seventh Framework Programme (FP7/2007-2013) under grant 482 agreement n° [214478], project name NanoReTox.

N°P4c-2

COMBINED EFFECT OF ZnO NANOPARTICLES ON ZEBRAFISH EARLY DEVELOPMENT

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Although there have been a number of studies on the potential hazards of metal oxide NPs to aquatic ecosystems, their combined impact with other xenobiotics on aquatic organisms still have not been known. Available data has indicated that nZnO were acute toxic for aquatic species. It was shown that nZnO induce malformation and blocking hatching to zebrafish embryos. In this study, we examined the combined toxic effect of nZnO and antibiotics solution on early development of zebrafish (*Danio rerio*). Zebrafish embryos were exposed either in control, 50 nm-nZnO (10 mg/l), antibiotics (10 and 100 mg/l) or mixture of antibiotic with nZnO. Three different antibiotics were used in experiment: tetracycline, erythromycin and kanamycin.

No significant effect on mortality of embryos was found for nZnO alone in concentration used. Rate of developmental abnormalities increased significantly up to 12.5% and rate of hatching embryos decreased up to 38.9% in nZnO alone exposure. Mortality and rate of developmental abnormalities were increased dose-dependent in antibiotic alone exposure. Only tetracycline in concentration 100 mg/l was acute toxic for embryos. It caused 89.6% of mortality and 85.4% of malformed embryos.

Combination of nZnO with all antibiotics except tetracycline in high concentration didn't influence on mortality of embryos. Rate of mortality significantly decreased in mixture of NPs with tetracycline (100 mg/l) up to 20.8%. Rate of developmental abnormalities significantly increased in mixture of nZnO with erythromycin (12.0%) and kanamycin (27.0%). At the same time it significantly decreased in mixture with tetracycline (100 mg/l) comparing with tetracycline alone.

In conclusion, nZnO were not only toxic for early development of zebrafish embryos but also caused combined effect with antibiotics. Combination of nZnO with erythromycin and kanamycin induced developmental malformation and significantly decreased hatching rate in embryos. However, mixture nZnO with tetracycline decreased toxic action of antibiotic. This work was funded by RFFI 10-04-00848-a.

N°P4c-3

EFFECT OF METAL OXIDE NANOPARTICLES ON EMBRYOTOXICITY OF DOXORUBICIN

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Metal oxide nanoparticles have the potential to remove contaminants from the aquatic environment. Because of their large surface:volume ratio metal oxide nanoparticles could adsorb contaminants from the environment. Nanoparticle-mediated degradation of drugs also plays an important role in removal process. The widespread use of antibiotics has resulted in the contamination of natural waters with drug compounds. It is important to understand the consequences of presence both metal oxide nanoparticles and antibiotics in the aquatic environment for living organisms.

The study investigated how presence of metal oxide nanoparticles effect on doxorubicin toxicity to zebrafish (*Danio rerio*) early development. It is known that doxorubicin has an embryotoxic action on fish development. Embryonic *Danio rerio* were exposed in a series of experiments to either control, metal oxide nanoparticles (10 mg/l), doxorubicin (1, 5 and 10 mg/l) or mixture of nanoparticles with doxorubicin. Anatase (> 25 nm) and rutil (> 100 nm) TiO₂, CeO₂ (> 25 nm) and ZnO nanoparticles were used in experiments. All experiments were performed in a dark condition in order to exclude phototoxic action of metal oxide nanoparticles and photocatalytic degradation of doxorubicin.

No significant growth mortality was found in nanoparticles alone or doxorubicin alone exposure in concentrations used. No significant teratogenic effects of metal oxide nanoparticles alone or doxorubicine alone occurred with the exception of nZnO. The rate of developmental abnormalities significantly increased up to 9.7% in nZnO exposure comparing to control (2.8%). Embryos exposure in mixture anatase or rutil TiO₂ with doxorubicin didn't influence on zebrafish early development. The rate of developmental abnormalities significantly increased in combination of ceria nanoparticles with doxorubicin in concentration 10 mg/l up to 50.0%. Mixture of nZnO with doxorubicin (1, 5 and 10 mg/l) dose-dependent increased rate of developmental abnormalities of embryos (22.2, 29.2 and 55.6% respectively). Thus, presence of metal oxide nanoparticles influenced on doxorubicin toxic action to zebrafish development even in absence of UV light.

N°P4c-4

**EVALUATION OF MULTI WALLED CARBON NANOTUBES ECOTOXICOLOGY USING
STANDARDIZED PROCEDURES ON AQUATIC ORGANISMS**

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Carbon nanotubes (CNTs) are a category of nanomaterials with unique chemical and physical characteristics. Therefore, they can be used to develop numerous potential applications, explaining their growing annual world production capacities. In this context, it is likely that some of them may contaminate the environment during their life cycle (production, use and disposal), especially in the aquatic compartment which is well known to concentrate pollutants. Present knowledge concerning their ecotoxic effects is rather limited due to their specific properties and deserves to be documented more extensively. Aquatic ecotoxicity assessment of CNTs is thus a challenge.

In this context, the potential ecotoxic properties of industrial Multi Walled Carbon NanoTubes (MWNTs, Graphistrength C100), synthesized by ARKEMA France, was investigated in the framework of the joint research laboratory NAUTILE (Nanotubes & Ecotoxicology). The approach consisted in using a test battery to screen potential effects of MWNTs in aquatic organisms, according to conventional and standardized bioassays and procedures. Such test batteries are usually carried out to evaluate the ecotoxicity of a new substance in the European framework of existing regulations for chemicals (REACH, CLP).

Aquatic organisms of different trophic levels used were decomposers (bacteria), primary producers (green algae, *Pseudokirchneriella subcapitata*), primary consumers (invertebrates *Daphnia magna* and *Crangon crangon*) and secondary consumers (fish and amphibians, *Danio rerio* and *Xenopus laevis*). Bioassays were carried out according to standardized protocols (OECD, ISO). This test battery covers several ecotoxicological endpoints measuring acute, chronic and genetic toxicities.

Results show that effects of raw MWNTs are weakly marked and expressed at unrealistic nominal concentrations. The results indicated (i) no toxicity in fish and daphnia up to 100 mg/L, (ii) inhibition of growth in amphibian from 50 mg/L of MWNTs, (iii) toxicity in the marine shrimp at 1g/L, (iv) inhibition of growth in algae at 120 mg/L. Excepted for alga (for which deprivation of iron as micronutrient through adsorption is suspected), it seems that the observed toxicity may be due to physiological effects in relation with MWNTs ingestion, and not necessarily related to their intrinsic toxic effects.

N°P4c-5

ADVERSE EFFECT OF SURFACE MODIFIED NANOPARTICLES ON THE AQUATIC ENVIRONMENT

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Surface modification is a popular technology adopted to overcome the weakness of nanoparticles by increasing biocompatibility, stability or chemical resistance or by changing surface charge potential and reactivity, which, however, increases concerns on the adverse effect of surface modified nanoparticles when they are discharged into aquatic environment.

In the present study, we investigated four types of nanoparticles coated with different techniques, where nanoparticles bind through physical and chemical mechanism and reveals hydrophilic and amphiphilic characteristics. Silver nanoparticles with citrate, polyethylene glycol (PEG) and bovine serum albumin (BSA) coatings are purchased and used in the study.

Bioassays are performed using MARA screening system containing 11 genetically diverse microorganisms (10 prokaryotes and 1 eukaryote). Microorganisms are exposed to nanoparticles with serial dilution for 18 hr at 30 °C and investigated with growth rate. As a result, MTC (microbial toxicity concentration) and EC₅₀ values are calculated for each microorganism. Further fingerprints of nanoparticles are to be investigated to give specific identification information of nanoparticles according to their coating characteristics.

N°P4c-6

APPLICATION OF MARA ECO-TOXICITY TEST SYSTEM TO NANO-SAFETY ASSESSMENT

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MARA test system is a toxicity screening tool containing 11 genetically diverse microorganisms. Due to the number of test organism and versatility in application to wide range of samples, MARA system is very favorable to the application of environmental toxicity tests. However, regardless of the benefit of MARA system, it rarely shows systemic application to nanoparticles.

In the present study, we investigated the feasibility of MARA system to surface modified silver nanoparticles such as citrate, polyethylene glycol (PEG) and bovine serum albumin (BSA). Microorganisms freeze-dried in a 96 well-plate are simply activated by adding reconstitution solution. Growth rate of the microorganisms is measured by measuring the thickness of reduced dye using ELISA reader and flatbed scanner. As a result, dose-response curves were developed to estimate EC50 values of each nanoparticle.

During the test procedure, sensitivity, reproducibility and precisions of the MARA system are evaluated to validate test method. Nanoparticles are carefully monitored to estimate the aggregation during the exposure using UV spectrum and scanning electron microscope (SEM), which suggests the stability of nanoparticles caused by interaction with reagents or dilution.

N°P4c-7

ACUTE AQUATIC TOXICITY OF PHOTOACTIVE TiO₂ NANOPARTICLES ANCHORED ON KAOLINITE MATRIX TO FRESHWATER GREEN ALGAE

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Due to the growing utilization of titanium dioxide (TiO₂) based nanomaterials a question related to potential environmental risks posed by these materials arises. These nanomaterials are often used for many different environmental cleaning technologies, especially water purification purposes. Therefore, a model TiO₂/kaolinite nanocomposite with TiO₂ weight content of 60 % was prepared and evaluated in non-calcined and calcined form in term of acute aquatic toxicity to freshwater green algae strains (*Desmodesmus subspicatus*, *Chlorella vulgaris*).

The nanocomposites were prepared using a hydrolysis of titanyl sulphate in the presence of kaolin SAK47 and calcined at 600°C due to desired transformation of the kaolinite to metakaolinite. Titanium dioxide nanoparticles were present in the anatase form confirmed by X-ray diffraction (XRD) analysis and Raman spectroscopy. The non-calcined sample contains TiO₂ in almost amorphous form contrary to the calcined sample. The anatase crystallite size was for both nanocomposites calculated based on XRD data. Non-calcined and calcined samples contain polycrystalline TiO₂ nanoparticles in size of several tens of nanometers, which consist of crystallites of 7 and 19 nm in size, respectively. Both nanocomposites were previously confirmed to have photocatalytic properties after UV irradiation according to the discoloration of aqueous solutions of organic dyes.

Toxicity assessment was performed according to the OECD 201 methodology: Test for the inhibition of growth of algae after 72 hour exposure including irradiation simulating daylight. The bioassay is based on the monitoring of freshwater algae growth inhibition of algal strain by tested material. Direct toxicity testing of the solid samples in suspension as well as evaluation of aqueous leachates were performed. Aqueous leachates of the studied samples were prepared according to the EN 12457 – 4 standard and suspensions were prepared in different weight concentrations. Algal cell density was measured using light microscopy and the counting chamber. According to the experimental data obtained the aqueous leachates of both nanocomposites caused no algal growth inhibition. This finding may be associated with tightly anchored TiO₂ nanoparticles on clay matrix what was confirmed by AES-ICP analysis detecting no titanium in the leachates. On the contrary, suspensions of both samples were found to have inhibitory effect on algal growth even at weight concentrations of 5 ‰ and the inhibition effect was increasing with growing sample concentration.

Despite the fact that TiO₂ based nanomaterials are very promising photocatalysts, they may pose potential risks to freshwater phytoplankton species responsible for oxygen production.

The study was supported by the projects MSMT LH12184, CZ.1.07/2.3.00/20.0074, SP 2012/45, SP 2012/65 from the state budget of the Czech Republic.

N°P4c-8

MONITORING TECHNIQUE FOR THE ACUTE EFFECT OF TiO₂ NANOPARTICLES FROM THE BEHAVIOUR OF RICE FISH *ORIZIAS LATIPES* IN VIVO.

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We have developed a monitoring technique to find the acute toxicological effect on the movement of aquatic organisms, with the response time from one tenth of a second to 12 hours. By applying this, we measured the effect of the exposure of anatase type <25nm TiO₂ nano-particles, with a surface area of about 50m²/gram on the behavior of rice fish *Orizias latipes*.

In this demonstration, 140 rise fish are divided into four groups and each fish is put in the tap water cell of 7mmL x 40mmW with a depth of 15mm. After one hour, hyper-sonically agitated TiO₂ water suspensions were added to make 0 ppb (control), 10ppb, 100ppb and 1ppm TiO₂ concentration. The movement of rice fish is monitored for 7 hours with 50 x 288 pixel camera with the frame rate of 25 frames/sec. The total records are 630 thousand frames as a whole. With an image processing technique focused on the gill deformation frequency of about 4Hz, the global movement is extracted with three dimensional Fourier transformation technique for every 128 frames (5.12s), Fig.1 and Fig.2. The analysis take less than 6 hours with a conventional personal computer with GPGPU (General Purpose Graphical Processor Unit). The velocity distributions of fish movement within exposure time of 6 hours are shown in Figure.3. These profiles averaged over 35 fish show no clear tendency of the nano-particle dose response. The detailed analysis depending on the size of each fish is in progress.



Fig.1. Image identification of the gill in rice fish.

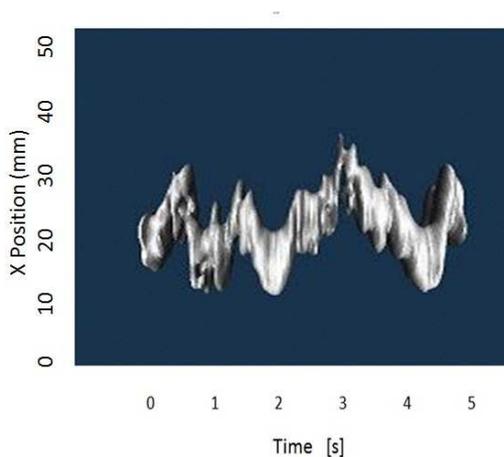
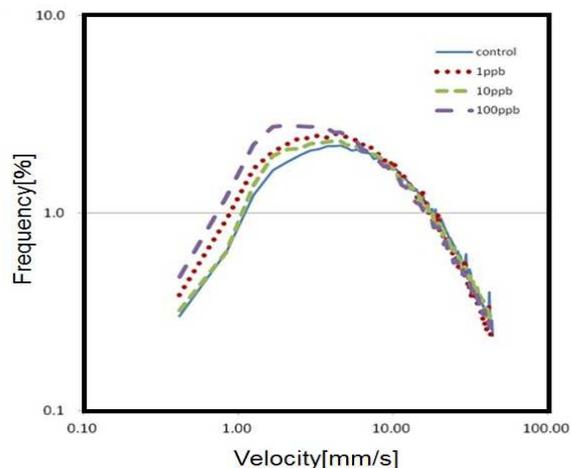


Fig.2 The extracted movement of the gill



Fif.3 The velocity distribution of the ricefish

N°P4c-9

ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF SILVER NANOPARTICLES

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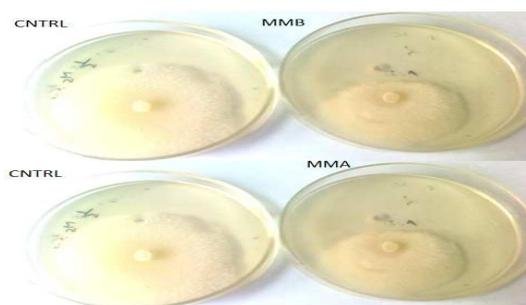
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Silver and its compounds have been reported to have broad-spectrum anti-microbial activity since ancient times. Number of chemical form of silver is known to exhibit antimicrobial activities against bacteria, fungal and viral agents. Because of low toxicity of silver nanoparticles to human cells its long lasting activity microbes have received great attention in various biomedical application e.g. antimicrobial paints, wound dressings based on sputtered nano-silver are employed in hospitals to suppress microbial infection in burn wounds and cryo protective activities towards human deficiency viral 1 infected cell has also been reported. In our studies we have prepared a different shape and size of Silver Nanoparticles of diameter less than 100nm were prepared in aqueous medium at room temperature and applied as a therapeutic agent against *Malaseezia furfur* fungus which is a dandruff causing agent and against gram positive (*Staphylococcus aureus*) and gram negative (*Pseudomonas* sps.) bacteria.



*Fig.1. Antifungal activity of MMA and MMB
Against Phytophthora*

These nanoparticles were characterized using UV-Visible, FTIR spectroscopy, TEM and XRD techniques. The Hemolytic toxicity of these nanoparticles were also carried out which proved that these nanoparticles are less toxic as compared to the conventional drug ketoconazole which is used to kill the dandruff. And also have potential to fight against bacterial strength.

Keywords: Silver nanoparticles, Antimicrobial, Staphylococcus aureus, Malaseezia furfur, Pseudomonas sps.

N°P5a-1

CARBON NANOTUBES SORBENTS AND THEIR POTENTIAL RISKS TO AQUATIC ORGANISMS

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Carbon nanotubes (CNTs) are very promising nanomaterials for 21st century. They exhibit unique structural, mechanical and electrical properties. For that reason they can be applied in many industrial applications, especially in polymer nanocomposites, nanoelectronics, supports in catalysis, hydrogen storage media, energy conversion devices, nanodevices, nanobiosensors, nanobioelectronics, nanomedicine, selective gas separation, and environmental remediation. Due to their structural and surface properties, they also seem to be an ideal sorbent material for inorganic and organic pollutants. Their properties are comparable with activated carbon due to their chemically inert surfaces; however, their structure at atomic scale is more well-defined and uniform with defined adsorption sites. There are many research studies that have confirmed that CNTs are very effective adsorbents for inorganic pollutants such as metals and also organic pollutants, radionuclides and biological agents from gas stream and polluted water.

Commercially available sample of CNTs was assessed in term of sorption of mono- and polycyclic aromatic compounds and their derivatives. Within the study unique properties of this material regarding the adsorption ability for organic compounds were confirmed. Special attention was paid to the dynamic sorption set up, but also the static sorption was performed. Nevertheless, utilization of these nanomaterials in water environments may be associated with unintentional release of carbon nanotubes into the water ecosystem. Therefore, acute aquatic toxicity to two freshwater green algae strains (*Desmodesmus subspicatus*, *Chlorella vulgaris*) of aqueous solutions/suspensions after successful purification was evaluated. According to the experimental data it is obvious that both processes utilizing CNTs are associated with presence of water-suspended carbon nanotubes in the purified solutions which was confirmed by Raman microspectroscopy. Furthermore, these purified solutions were found to cause inhibition of algae growth, i.e. phytotoxicity. The effect observed may be caused by released CNTs but also by water soluble impurities in initial CNTs sample.

The study was supported by the projects MSMT CZ.1.07/2.3.00/20.0074, CZ.1.05/2.1.00/03.0069, SP 2012/65 from the state budget of the Czech Republic.

N°P5a-2

ECOLOGICALY FRIENDLY INTERMATRIX SYNTHESIS OF POLYMER STABILIZED SILVER NANOCOMPOSITES: DEALING WITH NANOMATERIAL RELEASE.

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Nanotechnology is one of the hottest topics nowadays. However, their effects are still unknown. For this reason, nanosafety studies are becoming more interesting, resulting in the formation of specialized research groups (p.e. Nanosafety Cluster¹⁰)

The main concerns related with the presence of nanomaterials (NMs) in different media, for example in water or air, are associated with the following facts:

- 1) the higher toxicity of many NMs in comparison with the corresponding bulk materials due to their higher reactivity
- 2) the absence of adequate analytical techniques for detection of NMs, and
- 3) the lack of the legislation related with the allowed levels of NMs, directly related with their still unknown effects.

One possible solution to avoid the leakage of metallic nanoparticles (NPs) to the media, is the Intermatrix Synthesis (IMS) as a way to stabilize NPs into a polymeric matrix. Moreover, it could be combined with the formation of magnetic NPs that could be easily trapped by using a magnet, for instance core-shell NPs formed with a superparamagnetic core coated with a functional metal shell of minimal thickness (to decrease their cost). Therefore, this nanomaterials provides an additional level of the material safety, due to NPs are not only stabilized but also, in case that NPs were leached from the polymer matrix, they can be easily captured by magnetic traps to completely prevent any post-contamination, for example, of the treated water. In addition, in the case of catalytic applications of NMs this will allow the reuse or the recycling, particularly important in case of noble metals NPs due to their high cost.

Finally, it is noteworthy that the functional groups are not blocked after the NPs formation in IMS. So that the polymer-metal nanocomposite can be also used for the removal of some undesired ions (e.g. hardness ions, iron, nitrates, etc.), which is particularly important in the case of complex water treatment.

Nanomaterial release was studied in the case of silver nanocomposites obtained by IMS on Purolite® C100E sulfonic ion exchange resin, performed in order to support the stability of the novel material obtained. After mechanical stirring for different periods of time no significant morphological changes were observed but the outbreak of the polymer layer which contains de silver NPs. This supports the idea of the NM being stabilized on the polymer surface and most of them are not released to the surrounding environment.

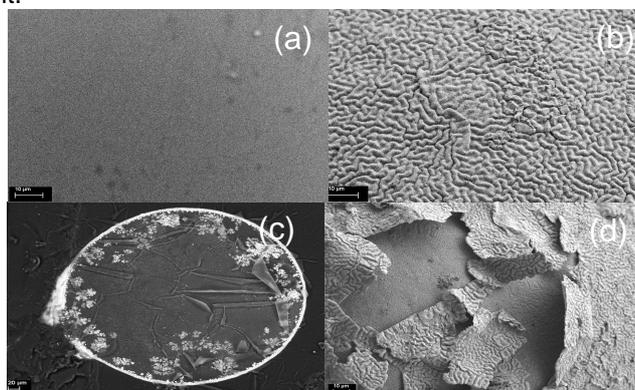


Figure 1: SEM images of a) Non modified ion exchange resin b) modified ion exchange resin with Ag nanoparticles, c) cross section of Ag nanocomposites and d) outbreak of Ag Nanocomposite surface.

¹⁰ <http://www.nanosafetycluster.eu/>

N°P5a-3

CHARACTERIZATION OF NANOPARTICULATE EMISSIONS FROM THE INCINERATION OF WASTES CONTAINING MANUFACTURED NANOMATERIALS

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In a perspective of sustainable innovation, the development of nanotechnologies requires a deep insight in the nanosafety during the whole life cycle of products containing nanomaterials, from production, to recycling and final destruction. Up to now there is no peculiar approved procedure for the waste management of nanoobjects at their end of life, mainly because the current regulation does not consider the typical nano-specificity of such emerging products.

The French project called NANOFIueGas, led by the INERIS, has recently started for a period of 3 years (2011-2014) to provide data and to examine nanosafety aspects during the final destruction of nanomanufactured products by specialized thermal treatment. This project is coordinated by the INERIS and benefits from the full competences and knowledges of both the ECOLE DES MINES DE NANTES-GEPEA laboratory, a main academic partner specialized in engineering, and TREDI-SÉCHÉ ENVIRONNEMENT, a major expert of hazardous wastes incineration in France. The main objectives of this project supported by ADEME are: (1) to better understand possible mechanisms of nanoparticle release in the raw gas during the combustion of different kinds of wastes containing manufactured nanomaterials and (2) to evaluate the efficiency of the current pollution control processes and procedures implemented to clean the flue gas on waste incineration facilities.

The experimental part is twofold. First, an insight will be provided on the emission mechanisms and on the characterization of nanoparticles in the raw smokes (particle number and size distribution, chemical composition, shape and morphology...) during the incineration at both pilot-scale and on industrial units. Secondly, the NANOFIueGas consortium will review the efficiency of main available pollution abatement processes, thus providing first fully adapted recommendations on procedures and technological features for the processing of nanowastes.

The initial works of the NANOFIueGas project were aimed at: (1) identifying possible sources containing nanomaterials; (2) selecting three representative nanomaterials; and (3) providing small-scale incineration devices closest to real-scale installations.

The purpose of this communication is to describe the different steps of the project and to present the results of the small-scale incineration of three types of nanowastes under different physical forms (liquid, structured solid, and bulk solid) in relation with the regulatory conditions and the key-parameters that pertain to full-scale waste incineration, *i.e.* chiefly *i)* the control of Temperature, residence Time and Turbulence in the combustion and *post*-combustion zones (the "3T" rule), and, *ii)* the large excess *air* requirement to ensure complete combustion of organics. Concretely, this work will discuss the characterization of gases (O₂, CO, CO₂, NO_x) and particles in the raw fume as well as the composition of ashes, in relation with different operational parameters (residence time, quantity and physical form of the nanowastes input, ratio of excess air...).

N° P5a-4

INVESTIGATION OF NANOPARTICLE RELEASE FROM UV-CURABLE POLYMERIC NANOCOMPOSITES

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Polymers modified with nanoparticles are intended to combine quite different properties, such as flexibility, hardness, ductility, and thermal stability. Therefore, such nanocomposite materials have successfully been applied in many fields in engineering industry as well as consumer products. During their lifecycle particle release due to weathering, aging or mechanical stress is possible. The analysis of such release and release products is necessary for assessing the potential exposure risks for consumers. Due to the stability of conventional industrial products only very small fractions of nanoparticles are released. Therefore accurate and sensitive detection is limited or requires an accumulation of release products for a long time period or investigation of large product samples to overcome this difficulty.

The application of radiotracers provides the unique option of “visibility”, traceability and quantification of a very small quantity of nanoparticles in complex media. For the investigations presented here radioactive labelled TiO₂ (P25, Evonik Industries, d_p = 21 nm) was used. Labelling was achieved by two different processes, either diffusive introduction of ⁴⁴Ti radionuclides (t_{1/2} = 63 a) or direct activation of the nanopowder via proton irradiation resulting in ⁴⁸V-labelled TiO₂ (t_{1/2} = 16 d) (Abbas et al. 2010, Holzwarth et al. 2012). The labelled TiO₂ was dispersed in a practical like UV-curable acrylate composition and a labile “worst case” – formulation with a pure acrylate. The nanocomposite formulations were applied on a substrate and UV-cured. Subsequently the samples were aged with UVA radiation. The treatment caused slow progressive damage of the varnish matrix and NPs can release from surfaces. The released products were transferred in an aqueous suspension and the radioactivity was measured. Parallel to these experiments tests were done with the same formulation, but without labelled TiO₂. For this control tests only commercial P25 was used. The suspension of the control experiments were analysed by conventional methods, also the coating themselves were investigated for example by FT-IR and SEM.

First results of these investigations will be presented on this poster.

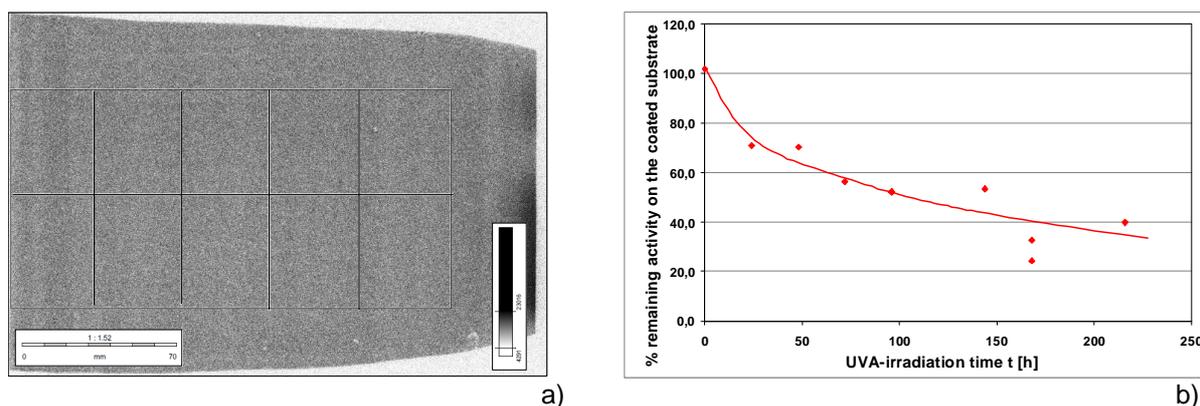


Figure 1: a) Autoradiography of a substrate, coated with polyacrylate TiO₂ nanocomposite with labelled TiO₂, b) Time-dependent degradation of a polyacrylate TiO₂ nanocomposite by UV-A-irradiation (intensity ~ 15 mW/cm²); measurement of activity on the coated substrate after transfer of the release products; Autoradiography: Helmholtz-Zentrum, Leipzig Site.

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N°P5a-5

**GENERATION OF AEROSOLS DURING THE MECHANICAL SOLICITATION OF MATERIALS:
DEVELOPMENT OF AN EXPERIMENTAL SET-UP AND APPLICATIONS**

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The overwhelming use of nanoparticles in contemporary applications has brought huge potential risks to the workers and consumers due to their release during their use. This release is usually in the form of polydispersed aerosols, from nano to micronic size range. Towards the safety concern of this release, in-vivo tests and analyses have been carried out (Kohler 2008, Wohlleben 2011) and still going on. Various exposure models have also been devised (Duuren-Stuurman 2011, Schneider 2011) but surprisingly very limited studies have been done concentrating on how and why they are being released.

The release of the aerosol particles is a direct result of the wear or fretting of surfaces when solicited mechanically. This wear solely depends on the parental contact conditions exhibiting various regimes of different stress states. These contact conditions have been studied (Greenwood and Williamson 1966, Kapoor et al. 1994, Jiang et al. 1998, Eriten et al. 2011 etc.) to explain different surface phenomena but no concern was given towards them getting airborne.

This study deals with the potential emission of particles in air. A linear abrasion wear mechanism is selected. The experimental device comprises of an emission chamber, a Taber type linear abraser, real time aerosol measuring devices (CNC, APS, SMPS), and a system for collecting the sample particles for their further microscopic analysis using TEM (R'mili 2011).

An initial parametric study has been carried out on a brick, to develop the prototype and to identify Taber key parameters leading to the generation of aerosols. The parameters studied include normal load, sliding speed, abrasion cycles and type of abrasive. This approach has been further applied to two products containing manufactured nanomaterials: pen and computer keyboard.

The results show that during abrasion of the brick, considerable emission of material occurs in the form of aerosol. The produced particles are principally in micronic size range with a nanometric fraction. That is not the case for pen and computer keyboard for which the emission in air gets inhibited by static triboelectric charge deposition during abrasion.

For pen, some elements of nanomaterials are observed in micronic size range, in which the composition is representative of the original matrix. The particles collected from the abrasion of computer keyboard enabled us to identify free particles of TiO₂ in nanometric size range.

Acknowledgements: the authors thank ANSES ("nanoEMIS" project) and French Environment Ministry (prg 181 DRC 33, prg 190) for financial support.

N°P5a-6

RELEASE OF NANOMATERIALS FROM PAINT WASTE

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The introduction of engineered nanoparticles (ENPs) into traditional paints for indoor and outdoor applications has been recently proposed. The paint industry already use ENPs in paint formulations, e.g. silver (Ag), titanium dioxide (TiO₂) and silica dioxide (SiO₂) ENPs, as additives for the protection of paints against microbial degradation and physical and chemical deterioration. Experts expect that, in the industrialized countries in 2020, over 15% of sector revenues will be generated by products containing ENPs (European Coatings Journal, 2009). Consequently, in the near future paint industry workers but also people may potentially become exposed to ENPs during production and application of these products, as well as during subsequent finishing and renovation. The uncertainty about the release, and the potential hazard, of ENPs into the environment from application, ageing or renovation of ENPs-based paints is one of the most important topics that should be investigated for a safety introduction of these new products. Unintentionally release of ENPs into the environment may occur during the whole nano-based paint's life-cycle. Within the EU-funded NanoHouse project (*Life cycle of nanoparticle-based products used in house coating*) aimed at developing appropriate solutions for a safe and sustainable use of nano-based coatings according to Life Cycle Thinking (LCT), the potential release of ENPs from paint waste has been analyzed. According to literature review, we still have very limited information concerning nano-based product's end of life, and the fate of the different ENPs when that products are discharged has not been yet investigated. Leaching tests play an important role in the characterization of waste, especially in relation to assessment of their potential environmental impacts when utilized or deposited. In our work, we analyzed the leachability of TiO₂, SiO₂ and Ag ENPs from three different paint debris in batch experiments as a function of liquid-to-solid (L/S) ratio, applying the standard UNI EN ISO 12457:2002 used for characterization of granular solid waste. In detail, plastic panels were coated with 0.3 kg/m² of three different paints containing the listed above ENPs. After drying in indoor ambient, paints were removed by plastic spatula and collected. Paint debris were sieved to obtain powders smaller than 4 mm. Paint powders samples were then placed in contact with demineralized water in a closed leak-proof bottle for 6 hours with L/S=2 l/kg (1st step), and 18 hours with L/S=8 l/kg (2nd step), i.e. a cumulative ratio of L/S=10 l/kg. Leaching liquids were collected and analyzed by using Transmission Electron Microscopy (TEM) and Inductively Coupled Plasma (ICP). Suspended and dissolved solids, pH, conductivity was also determined together with total Ti, Ag and Si. From results, 219.3 mg/l of Si was measured in leaching liquids (fig. 1). Bulk chemical analysis has been confirmed by TEM analysis, as first microscopy observation showed that aggregated SiO₂ NPs are released, together with other large particles (e.g. calcium carbonates). While little amount of Ag and Ti was measured (from 0.07 to 3 mg/l). In addition, results showed that L/S influences release, i.e. ENPs are released rapidly during the 1st step.

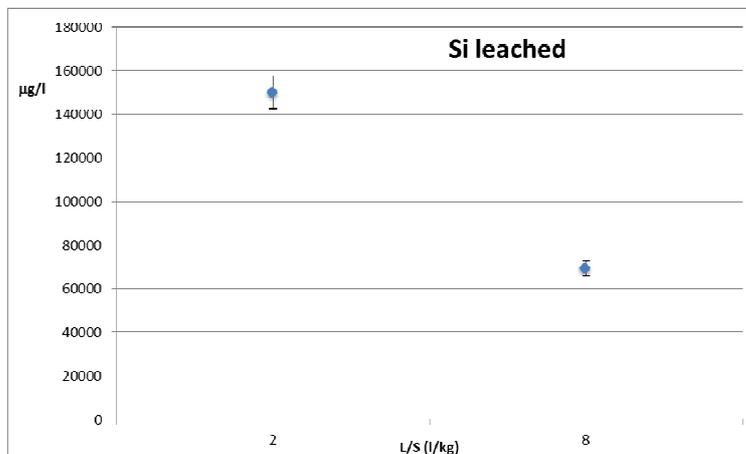


Figure 1. Si leached from paint debris as a function of L/S ratio.

N°P5a-7

POTENTIAL RELEASE OF CARBON NANOTUBES FROM CNT COMPOSITES DURING GRINDING PROCESS

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Carbon nanotubes (CNTs) have unique properties that make them an interesting prospect as filler material in composites. CNT composites are expected to be used in a wide range of industrial applications and consumer products. However, there is still a concern about the potential impact of CNTs on health. To perform a risk assessment of CNT composites, it is important to know the potential release of CNTs throughout the life cycle.

In this study, we investigated the release of particles caused by machining of polystyrene-based composites with and without single-wall CNTs (i.e., CNT-containing and CNT-free). The CNTs had a tube diameter of approximately 3 nm and were synthesized by a water-assisted chemical vapor deposition (CVD) method [1]. The CNT composites were ground by a micro grinder in a conductive/antistatic plastic box that had an air supply opening with a HEPA (high efficiency particulate air) filter. Before the test, purified air was passed through the box to eliminate background particles. The aerosol particles released into the box by the grinding process were measured using real-time aerosol monitoring instruments, such as a condensation particle counter (CPC), an optical particle counter (OPC), and a scanning mobility particle sizer (SMPS). Moreover, a thermodenuder [2] was used to distinguish CNT-containing particles released by the grinding process from volatile particles potentially released by the friction heat caused by grinding the composite. Released aerosol particles collected on Nuclepore membrane filters (pore size 0.08 µm), as well as particles deposited on the inner surface of the box and the grinding surface of the test composite, were observed by scanning electron microscopy (SEM).

In the results of real-time aerosol monitoring, considerable increases in the number concentration of nano-sized aerosol particles were observed during the grinding of CNT-containing composites. However, similar increases were also observed when CNT-free polystyrene was ground. When the thermodenuder was used, the number of nanoparticles released dropped almost to zero, indicating that the nano-sized aerosol particles were not CNT-containing particles. In the electron microscope analysis of aerosol particles, micron-sized particles with protruding CNTs were observed, whereas free-standing CNTs were not observed.

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N°P5a-8

NANOMATERIALS FOR CONTROLLED DRUG DELIVERY SYSTEM

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The development of nanotechnology brought generations of nanomaterials with specific biomedical properties. These include the nanoparticle-based drug delivery, nanosized drugs, and nanomaterials for tissue engineering. Carbon nanotubes (CNTs) are very prevalent in today's world of medical research and are being highly researched in the fields of efficient drug delivery and biosensing methods for disease treatment and health monitoring. The use of CNTs in drug delivery and biosensing technology has the potential to revolutionize medicine. Functionalization of SWNTs has proven to enhance solubility and allow for efficient tumor targeting/drug delivery. It prevents SWNTs from being cytotoxic and altering the function of immune cells. Controlled nanoscale drug delivery systems are more efficient, reduce toxicity, and are more convenient than traditional methods. Nanoparticles/nanomaterials can be manipulated to target areas of the body specified by the doctors. Once this technology transitions from the labs to the operating rooms it can bring hope and life to people dwindling at the edge of death.

N°P6a-1

**RELEASE CHARACTERISTICS OF SINGLE-WALL CARBON NANOTUBES DURING
MANUFACTURING AND HANDLING**

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Although carbon nanotubes (CNTs) have unique properties that make them potentially useful in many applications, there is a growing concern about their potential impact on health. At present, workers (and researchers) involved in CNT manufacture and handling are potentially exposed to elevated levels of CNTs, mainly through inhalation. Recently, occupational exposure limits of airborne CNTs have been proposed [1-3]. Information on airborne CNT release, such as the magnitude, size distribution, and morphology of airborne CNTs, is required in order to evaluate and control CNT exposure.

In our previous study [4], we conducted on-site aerosol measurements at a research laboratory where a relatively small amount of single-wall CNTs, which had a tube diameter of approximately 3 nm and formed superdense and vertically aligned nanotube forests [5], were synthesized and handled. Recently, a pilot-scale plant has started production of CNTs at a rate of 600 g/day and the supply of CNT samples has accelerated the development of new applications. In this study, we investigated the release characteristics of CNTs synthesized by the pilot-scale plant. In addition to on-site aerosol measurements at the pilot-scale plant where the CNTs are synthesized, harvested, and packed, we conducted dustiness (emission) tests by vortex shaking [6] and by transferring CNTs from one bowl to another (with a fall of about 30 cm).

The aerosol particles were measured using real-time aerosol monitoring instruments, i.e., condensation particle counters (CPCs), optical particle counters, and light-scattering laser photometers. For the vortex shaking dustiness test, a scanning mobility particle sizer and an aerodynamic particle sizer were also used. The aerosol particles were collected on Nuclepore membrane filters (pore size 0.08 µm) for scanning electron microscopy (SEM) analysis and on quartz fiber filters for carbon analysis (similar to NIOSH method 5040).

In the results of on-site aerosol measurements at the pilot-scale plant, slight increases in the concentration of aerosol particles were observed by the aerosol monitoring instruments when CNTs were harvested (<30 particles/cm³ with a CPC and <0.01 mg/m³ with a laser photometer). For filter samples collected during the harvesting process, elementary carbon was detected (but less than the quantitation limit) by carbon analysis (<0.002 mg/m³), and micron-sized CNT clusters were observed by SEM analysis. For samples collected during other processes, no elementary carbon was detected and no CNTs were observed. The results of dustiness tests revealed that submicron-sized particles were dominant in the number concentration measured by aerosol monitoring instruments, whereas micron-sized CNT clusters were mainly observed by SEM analysis. The lower drop impact of CNT clusters due to their lower bulk density resulted in lower CNT release from falling CNTs.

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N°P6a-2

**TOWARDS A NANOSECURED PLATFORM TO ASSESS RISKS ALONG THE
INDUSTRIAL LIFECYCLE OF NANOMATERIALS**

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Nanotechnologies, as drivers of technological innovation, bring new nanomaterials (NM) that may induce possible risks that have to be evaluated and managed to enable their sustainable and efficient development. This growing-fast technology and the recent results published in the literature put into evidence that a major effort is now required to assess the safety parameters of NMs, especially their ability to burn, explode and disperse in case of a loss of containment, in order to control the risks at industrial-level. Other parameters relative to the emission of NMs by end products at various stages of their lifecycle (wear, use, weathering, machining) are to be investigated. Such parameters are becoming critical to control the risks at industrial level, and have to be taken into account in the safety-by-design approach.

In this perspective, INERIS, whose mission is to support the development of safe nanotechnologies, is setting up a new platform for open research and expertise. Located on INERIS main site, Verneuil-en-Halatte (France), this infrastructure consists of several laboratories and associated facilities that will be fully operational in 2014. This nanosecured platform is dedicated to the metrology of nanomaterials and the characterization of their potential dangers, particularly in the context of the safety of industrial processes. Its major objectives are to:

- characterize and understand the intrinsic safety parameters to reduce accidental risks during processes (e.g. flammability, self-heating, explosivity, electrical charging);
- assess the release nano-objects from products throughout their lifecycle (e.g. machining, accidental fire, incineration);
- investigate the dustiness and the dispersion of NMs to assess the risk of chronic exposure and the impact of an accidental loss of containment (e.g. dispersion models);
- develop associated measurement methods and techniques to enable a safe, efficient and reliable operation of industrial units.

In the current absence of reliable data about toxicity and threshold limit values, it is a real challenge to setup safe experiments in which NMs on purpose put into high energetic processes, like the generation of dust explosion, dispersion in air or liquid for toxicity and environmental studies,), but also experiments in which products are voluntarily put into use or recycling conditions. Proper safety barriers and containment devices are being implemented so as to enable the safe use of safety test apparatuses initially designed for micrometric powders, such as the ones used to assess the consequences of accidental scenarios (fire, exposure) in the laboratory itself, without generating an accident.

From experimental observations, we will practically illustrate how this platform can help improving the safety of some typical industrial processes.

Acknowledgments: *This platform has being designed as an open research and innovation tool in Picardy and is one component of SERENADE, a National “Laboratory of Excellence” sponsored by the French Ministry of Research, which focuses on eco-design of nanomaterials in order to preserve the environment during their end-of-life recycling, and protect occupational health and end-of-line users. SERENADE is led by the University Paul Cézanne Aix Marseille III and involves INERIS. In addition, this facility is financially supported by the Regional Council of Picardy.*

N°P6a-3

**MAPPING THE USE OF NANOPARTICLES IN QUEBEC'S INDUSTRIES
AND RESEARCH LABORATORIES**

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Nanotechnology offers important opportunities for Quebec's economy. In Quebec alone, an estimated 2.000 people, in research and industry fields, are potentially exposed to nanoparticles.

It is therefore, important to continue research and prevention to better characterize exposure, in particular through a better understanding of nanoparticles (nature and quantity) manipulated by Quebec companies and research laboratories.

In 2007, NanoQuébec conducted a survey which led to the identification of researchers and producers involved in nanotechnology for the province of Québec, Canada. Since this survey, the users' profile has evolved significantly. In fact, today, many companies buy or generate nanoparticles in order to introduce them into their manufacturing processes.

This research aimed to map the state of nanotechnology in Quebec by compiling the type and volumes of nanoparticles used as well as gathering information related to the working conditions and occupational health and safety (OHS). This survey was conducted among 1300 Quebec industries and 653 university researchers working in different fields potentially involved in the use/production of nanoparticles. Questionnaires administered by the Survey Monkey platform were sent to participants after an initial telephone survey which purpose was to confirm the use or production of nanoparticles.

This work describes Quebec's users/producers of nanoparticles' profile in terms of human resources, activities, types of markets served / objects of research, training of workers involved with the nanoparticles, type and quantity as well as the source of nanoparticles, etc. The results demonstrate the diversity of nanoparticles used, but also show a lack of training in OHS. Moreover, OHS trainings are considered essential by the participants. Some examples of nanosafety & nanoprotection measures used in the concerned industries will also be presented.

N°P8a-1

LIFE CYCLE AND DESTINATION OF SILVER NANOPARTICLES IN ENVIRONMENT

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Although silver has been widely used by human as an antibacterial material since centuries ago, it has also been classified as a toxic and stable pollutant. Certainly, new and fast developments in manipulating materials in nano-scale have ended up with explosion of ideas and production of nano silver particles. The theory containing four sections of resource, line, receiver and effect (developed by Owen and Handy, 2007) could propose a suitable frame for the analysis of dangers caused by such productions specifically silver nanoparticles.

It seems that morphology and type of silver nanoparticles are influential in their destiny and the effects they may have on the environment. Nonetheless, trading organizations give incomplete mostly contradictory information about their products.

Eventually, the environmental dangers originated from silver nanoparticles depend on the type of particles, application, and speed of deterioration, destiny of metallic silver, bioavailability and toxicity of both metallic silver and nano silver particles.

In this study, Hazards and destiny of silver developed from new technologies in environment are evaluated.

Keywords:

Silver Nanoparticles, Toxicity, Environment, bioavailability, Ecotoxicology

N°P9a-1

TOWARDS NANORESPONSIBILITY

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The necessity for ethical governance of nanotechnologies comes from the fact that law and standard ethical processes (like academic ethical committees) are not sufficient to address issues like health and environmental risks, impacts on liberties, privacy, security, justice and above all social values issues and priorities (usefulness, public interest, responsibility, sustainability, fairness) or other emerging ethical issues (how to deal with new uncertainties?) associated with the nanotechnological products and their design.

What should ethical governance of nanotechnologies look like? It is now recognized at the international level that one of its pillars is the stakeholders dialogue: civil society and NGOs have to be involved in the technological development, as early as possible, along with the scientific and technical communities and the policy makers in order to path the way for responsible innovation that creates social value.

Our organization, VivAgora, is developing the tools and experimentations for inclusive and cooperative governance following 7 principles:

- Information tools: “giving a hold” over the issues

Connecting science and society begins by providing accessible reviewing of the scientific and policy literatures to enable the actors to “have a hold” over the relevant issues and controversies (example: Alliance ACEN).

- The pluralist expertise: “giving meaning”

Every consultation or dialogue process, as well as its organization committee, should comprise all the possible stakeholders, including those who have demonstrated their opposition to the technologies (process experienced: COEXnano)

- Durability: long-lasting dialogue to “give weight”

It is important to make possible long-term processes like forums where the discourses of civil society can meet those of other stakeholders in order to make emerge, discuss and evolve not only technical points but also societal and ethical perspectives and concerns. VivAgora has experienced such processes like the “Nanoforum” (with the CNAM) and “Xenop”.

- Co-construction

The specific topics of the dialogue should not be predetermined; they should be collectively decided on and co-constructed throughout the dialogue process. The critical uncertainties can also be identified during the process.

- Denying acceptability

Setting the acceptability of the technologies as the purpose of the dialogue undermines its legitimacy and its democratic principles. A social contract is the first condition for building confidence.

- Be productive for innovation’s teams

The process should be aimed at producing a “map” of the issues according to 3 lines that support responsible innovation:

- Are the products conceived in a responsible manner (preservation of natural resources, low energy and carbon impacts, social equity);
- Is the life cycle of the products well-controlled and assessed (safe by design)?
- Is the marketing of the products transparent with well-designed information along the chain from producers to end-users?

- Guarantor

A personality recognized for his/her involvement in ethical issues plays the role of external guarantor and makes sure that the process complies with the above principles of plurality, durability, freedom of speech, etc.

By such a methodology, it is neither recommendations nor consensus that will arise from such processes; the outcome will however be acceptable and reasonable options that take into account the concerns and particular perspectives of the different stakeholders.

N°P9a-2

NANOSAFETY - RISK GOVERNANCE OF MANUFACTURED NANOPARTICLES -- CHALLENGES OF SUBSTANCE REGULATION UNDER SCIENTIFIC UNCERTAINTY

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Manufactured particulate nanomaterials (MPN) are expected to be a major opportunity for the economic and sustainable development of many countries. Due to nanosize (app. 1 - 100 nm) these materials exhibit completely new or improved properties compared to larger particles of the same material, which makes MPNs attractive for the industry for applications in various branches. Thus, a wide range of MPN-containing products are on the market and many more are under development. While on the one hand nanoproducts promise innovation and sustainability, on the other hand the concern rises that the new properties of the MPNs within them bear also new environmental, health and safety (EHS) risks, which was even confirmed by some toxicological studies. However, there are still the great uncertainties regarding the actual health and environmental effects of MPNs as well as numerous methodological challenges to established risk assessment procedures (including definitions, analytics, exposure, life cycle and hazard assessments, etc.). At the same time, precautionary regulatory action with regard to MPN is demanded by a number of stakeholders and parts of the general public. Regulation under uncertainty raises the fundamental political question: "How lawmakers should regulate risk in the face of such uncertainty?"

To address this question in regard to scientific policy advice in the frame of technology assessment, we discuss three main challenges that have to be approached in this context: (1) The limitations that research on potential EHS risks of MNPs is currently facing plus the consequences for risk assessment and how to handle this. (2) The lack of a harmonized and generally accepted definition and delimitation of MPNs for regulatory purpose. (3) The intricacy to identify and finally choose suitable regulatory means and/or options of parliamentary action.



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