Leaching potential of nanomaterials during different human contact scenarios and end-of-life

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PLATE-FORME NANO SÉCURITÉ

BOOK OF ABSTRACTS

nanoSAFE'14

4TH INTERNATIONAL CONFERENCE ON SAFE PRODUCTION AND USE OF NANOMATERIALS

MINATEC, GRENOBLE, FRANCE

November 18-20 2014

MINATEC Grenoble France

Book of Abstract

INTERNATIONAL CONFERENCE ON SAFE PRODUCTION AND USE OF NANOMATERIALS

MINATEC, GRENOBLE, FRANCE
18-20 November 2014

NANOSAFE 2014

CONFERENCE ORGANIZATION

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Frederic SCHUSTER (CEA, FR)
Jean Francois DAMLENCOURT (CEA/PNS)
Vanessa GAULTIER (CEA/PNS)

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Welcome from the organizers

The issues of fast progress in the field of Nanosafety are up to the potential benefits that nanotechnology can bring to mankind. Making more efficient - more sustainable - easier to share mineral resources, increasing the yields of new energy technologies, enabling drugs that act selectively and locally are just few examples of the wide range of nanomaterial applications that currently benefit humanity.
Nevertheless, the dynamic development of nanomaterials requires the adhesion from the general public who rightly demand major progresses in Nanosafety as a prerequisite.

This is our exciting responsibility and challenge!

Following the successful outcome of the three past international conferences on **Safe production and use of nanomaterials: Nanosafe 2008, 2010 and 2012,** the organizing committee has the pleasure to welcoming you again to Minatec, Grenoble with some of the most famous specialists in the field.
This year, two new topics have been added dealing with the “New application of Nanomaterials” and Nano-responsible Development” in addition to the usual issues addressed in previous Nanosafe conferences such as Expology, Detection and Characterization, Toxicology, Environmental Interactions, Nanomaterials Release, Life Cycle Analysis, Regulation and Standardization, Risk Management.

The debates in 2012 proved highly successful so this formula has been kept with 3 round tables: **Nano-Responsible Development, Risks and Benefits for the Environment, Toxicology Progress.**

2014 is a great year for the organizing committee as we ramp up the Nano Safety “PNS” platform at Minatec, with a brand new building spread out over 2000 m^2 of laboratories and more than 50 doctors and engineers dedicated to Nanosafety.

We hope that you will enjoy this new Nanosafe edition!

The Nanosafe2014 organisers


Francois TARDIF


Frederic Schuster


Jean Francois Damlencourt


Vanessa Gaultier

Monday	Tuesday	Wednesday	Thursday
	7:45 Registration 8:30 Auditorium Welcome from the Organizing Committee	08:00 Auditorium - Session 4: Toxicology 8:45 Auditorium Session 4 8:45 Room B Session 3 8:45 Room C Session 2 8:00 Scaffold Petit Salon « closed event »	08:00 Auditorium -Session 7 8:45 Auditorium Session 7 8:45 Room B Session 6 8:30 Room C NanoDiode 8:30 Petit Salon Charac
	10:30-11:00 Coffee break	10:00-10:30 Coffee break	10:00-10:30 Coffee break
	11:00 Auditorium Session 1: New applications of nanomaterials 11:45 Auditorium Session 1	10:30 Auditorium Session 5: Environmental interactions of nanomaterials 11:15 Auditorium Session 5 11:15 Room B Session 3 11:15 Room C Session 4 10:30 Scaffold Petit Salon « closed event »	10:30 Auditorium Session 8: Life Cycle Analysis 11:15 Auditorium Session 8 11:15 Room B Session 7 10:30 Room C NanoDiode 10:30 Petit Salon Charac Meeting 10: 30 S 225 Session 4
	13:00-14:00 Lunch	12:30-13:30 Lunch	12:30-13:30 Lunch
	14:00 Auditorium Session 2: Exposure 14:45 Auditorium Session 2 14:45 Room B Session 1 Room C Session 12 Nanoresponsible Development	13:30 Auditorium Session 6: Nanomaterials release 14:15 Auditorium Session 6 14:15 Room B Session 5 13:30 Room C Session 4 13:30 Scaffold Petit Salon « closed event » MARINA	13:30 Auditorium Session 11: Risk Management 14:15 Auditorium Session 11 14:15 Room B Session 8 14:15 Room C Session 3 13:30 Petit Salon Session 9 Regulation 15:15 Debate 13:30–14:00 S 225–
	16:00-16:30 Coffee break	15:30-16:00 Coffee break	15:30-16:00 Coffee break
Registration	16:30 Auditorium Session 3: Detection and Characterization 17:15-18:00 Auditorium Session 3 17:15-18:00 Room B Session 2 16:30-17-30 Room C Debate Responsible development 17:30 18:30 Room C Debate Environment 18:30-21:00 Petit Salon Harmonization	16:00-18:00 Auditorium Session10: Commercial Equipement 16:00-17:00 Room B Session 5 17:00-18:00 Room B DebateTox. 16:00 Room C Session 6 MARINA	16:00 Auditorium Session 11 17:00 End of the Conference – Conlusion
	18:00-21:00 Grand Salon Poster Session	20:00-23:30 Cocktail Party-Château de Sassenage	

Monday 17 November 2014

16:30-19:30: Registration

Conference opening

Tuesday 18 November

MINATEC AUDITORIUM

(Chair: Georgios Katalagarianakis)

8:15-8:30	Welcome by the Organizing Committee **François Tardif** (CEA, PNS, France) and Jean- François Damlencourt (CEA, PNS, France)
PL0a 8:50-9:10	Nanosafety research policy under the EU H2020 programme Georgios Katalagarianakis (European Community, Belgium)
PL0b 9:10-9:30	Nanomaterials economy: future trends and forecast Tim Harper (Cientifica Plc, UK)
PL0c 9:30-9:50	Today and Tomorrow: nanoproducts available on the market Todd KuiKen (Wilson Center, U.S.A)
PL0d 9:50-10:10	Challenges and promising strategies for fabricating and using nanomaterial-enabled membranes for water treatment Mark Wiesner (Duke University, CEINT, USA)
PL0e 10:10-10:30	From nanomedicine to nanosafety: a journey into nanocharacterization Patrick Boisseau (CEA Leti, France)
10:30-11:00	*Coffee-break*

Session 1: New applications of nanomaterials

Tuesday 18 November

MINATEC AUDITORIUM

(Chair: François Tardif)

PL1
11:00-11:35
Nanoparticles properties and interest for industrial applications
François Tardif (Univ. Grenoble Alpes, PNS, CEA, France)

O1a-1
11:45-12:00
Nanoparticles: potential additives for sustainable lubrication
Fabrice Dassenoy (Ecole Centrale de Lyon – Laboratoire de Tribologie et Dynamique des Systèmes, France)

O1a-2
12:00-12:15
Prospects and potential safety implications of nanoformulation of agrochemicals in crops production
Cui Haixin, X. Zhao (Institute of Environment and Sustainable Development in Agriculture, The Chinese Academy of Agricultural Sciences, China)

O1a-3
12:15-12:30
Nanomaterials as a New Approach to Fire
Fiona Hewitt, D. Suleiman Eid Rbehat, A. Witkowski, A. Stec and T.R. Hull (University of Central Lancashire, U.K)

O1a-4
12:30-12:45
Super-strong nano-composite materials for bunker & command post in army
Dalvinder Singh Grewal (Desh Bhagat University)

O1a-5
12:45-13:00
The in vivo activation of persistent nanophosphors for optical imaging of vascularization, tumours and grafted cells.
Cyrille Richard, T. Maldiney , A. Bessière, J .Seguin, E.Teston, SK. Sharma, B. Viana, AJ. Bos, P. Dorenbos, M. Bessodes, D. Gourier, D. Scherman (Université Paris- Descartes, France)

13:00-14:00
Lunch

ROOM B

(Chair: François Tardif)

O1a-6
14:45-15:00
Exploration of Activation Energy and Electrical Applications of Synthesized Al Doped ZnO Nanomaterials as Humidity/Gas Nanosensors
Misra Suneet Kumar, N.K. Pandey and V. Shakya (Sensors and Materials Research Laboratory, University of Lucknow, India)

O1a-7
15:00-15:15
Application of carbon nano-tubes (CNTs)/alkyd resin composites as anticorrosive coating
M. A Deyab (Egyptian Petroleum Research Institute, EPRI, Egypt)

O1a-8 15:15-15:30
In vivo study of novel nanocomposite for prostate cancer treatment
Camila Silveira, A. J. Paula, L. M. Apolinário, W. J. Fávaro, N. Durán (Chemistry Institute, UNICAMP, Brazil)

O1a-9 15:30-15:45
Preparation, characterization and tests of incorporation in stem cells of superparamagnetic iron oxide
Haddad Paula, T.N. Britos, L. Min Li, L. D´Souza Li (Exact and Earth Sciences Department, Universidade Federal de São Paulo, Brazil)

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

Session 2: Exposure

Tuesday 18 November

MINATEC AUDITORIUM

(Chair: Derk Brouwer)

2a. Methods and Strategies

PL2
14:00-14:35
Recent developments in human exposure assessment
Derk Brouwer (TNO, Risk Analysis for Products in Development, Netherlands)

O2a-1
14:45-15:00
Mass vs number-based exposure assessment to nanoparticles, a comparison of a personal sampler and monitors
Faure Bertrand, H. Dozol, A. Guiot, S. Clavaguera, A. M. Todea, C. Asbach (Univ. Grenoble Alpes, PNS, CEA, France)

O2a-2
15:00-15:15
Analysis and characterization of multivariate stochastic signals sampled by on-line particle analysers. Application to the quantitative assessment of exposure to noaa in occupational scenarios
Lopez de Ipiña Jesús, C. Vaquero, C. Gutierrez-Cañas, D.Y. H. Pui (TECNALIA, Spain)

O2a-3
15:15-15:30
Exposure scenario libraries as a tool for exposure assessment
Sánchez Jiménez Araceli, S. Rashid, G. Boulougouris, M. Van Tongeren, D. Brouwer, W. Fransman, C. Fito (IOM, UK)

O2a-4
15:30-15:45
Towards a strategy for engineered nanomaterials exposure monitoring in the workplace: a case study
Boccuni Fabio, R. Ferrante, S. Lavicoli (INAIL, Italy)

O2a-5
15:45-16:00
Biomonitoring to nanoparticle exposure: approaches for the development of indicators of exposure and effect
Caroline Desvergne, M. Dubosson, V. Mossuz, M. Lacombe, V. Brun (Univ. Grenoble Alpes, PNS, CEA, France)

16:00-16:30
Coffee Break

ROOM B

2b.Release

(Chair: Jean-François Damlencourt)

O2b-1
17:15-17:30
Occupational exposure to nano-tio2 in the life cycle steps of new depollutant mortars used in construction
Celina Vaquero, N. Gelarza, J.L. López de Ipiña, C. Gutierrez-Cañas, I. Múgica, G. Aragón, M. Jaen, R. Pina, I. Larraza , A. Esteban-Cubillo, D. Thompson, D.Y.H. Pui (TECNALIA, Spain)

O2b-2
17:30-17:45
Quantitative characterization of airborne particulate release during spray-can and spray-gun application of nanoparticle-doped coatings
Daniel Göhler, M. Stintz (Institute of Process Engineering, Technische Universität Dresden, Germany)

O2b-3
17:45-18:00
First development to model aerosol emission from engineering materials subjected to mechanical stresses
Neeraj Shandilya, M. Morgeneyer, O. Le Bihan (INERIS, France)

18:00-21:00 Poster Session

Wednesday 19 November

ROOM C

2c. Case Studies

(Co-Chair: Olivier Witschger)

O2c-1
8:45-9:00
Exposure to airborne nano-sized particles from ceramic milling processes
Ana Sofia Fonseca, M. Viana, N. Pérez, X. Querol, A. López, E. Monfort (Universidad de Barcelona/IDÆA-CSIC, Spain)

O2c-2
9:00-9:15
Occupational exposure assessment during high volume synthesis and subsequent handling of multi-walled carbon nanotubes
Kuijpers Eelco, C. Bekker, W. Fransman, A. Pronk, D. Brouwer, P. Tromp, J. Vlaanderen, R. Vermeulen, L. Goderis (TNO, Netherlands)

O2c-3
9:15-9:30
Assessment of Exposure to ENM during Manufacturing and Downstream Use of Pigments, Inks and Paints
Spankie Sally, A. Apsley, S. Steinle, A. Sanchez Jimenez, M. van Tongeren, E. de la Cruz, C. Fito (Institute of Occupational Medicine, UK)

O2c-4
9:30-9:45
Exposure assessment to noaa during mixing of nanomaterials powders
Elżbieta Jankowska, P. Sobiech, W. Zatorski (Central Institute for Labour, Poland)

O2c-5
9:45-10:00
Strategy for the lowering and the assessment of exposure to nanoparticles at workspace case of study concerning the potential emission of nanoparticles of lead in an epitaxy laboratory
Sébastien Artous, E. Zimmermann, D. Locatelli, S. Derrough, Paul-Antoine Douissard (Univ. Grenoble Alpes, PNS, CEA, France)

10:00-10:30 Coffee-break

Session 3: Detection and Characterization

Tuesday 18 November

MINATEC AUDITORIUM

3a. Detection

(Chair: David Y.H. Pui)

PL3
16:30-17:05
Measurement and filtration of air/liquid/surface-borne nanoparticles in support of sustainable nanotechnology
David Y.H. Pui (Distinguished McKnight University Professor, LM Fingerson/TSI Inc Chair in Mechanical Engineering, Director of the Particle Technology Laboratory, University of Minnesota, Minneapolis)

O3a-1
17:15-17:30
Towards an indicator of nanomaterial deposition in the human lung
Dimitrios Bitounis, C. Guibert, V. Forest, D. Boudard, J. Pourchez, J.M. Vergnon, M. Cottier (LINA / Pneumology and Histology-Cytology Departments, France)

O3a-2
17:30-17:45
Identification of Carbon Nanotubes by Thermal-Optical Analysis
Bertrand Faure, P. Babbar, A. Guiot, S. Artous, P. Tiquet, S. Clavaguera, S. Derrough, J.-F. Damlencourt (Univ. Grenoble Alpes, PNS, CEA, France)

18:00-21:00
Poster Session

Wednesday 19 November

ROOM B

(Chair: David Y.H Pui)

O3a-3
8:45-9:00
Experimental challenges for the detection of nanoparticles in food and cosmetic products
Retamal Marín Rodrigo Renato, F. Babick, M. Stintz (Technische Universität Dresden, Germany)

O3a-4
9:00-9:15
Detection of carbon nanotubes after an abrasion experiment
Lukas Schlagenhauf, A. Wichser, F. Nüesch, J. Wang (Swiss Federal Institute for Materials Testing and Research, Switzerland)

O3a-5
9:15-9:30
The use of small-angle x-ray scattering for the characterization of nanoparticles in biological matrices
Zoltan Varga, C. Gollwitzer, R. Garcia-Diez, M. Krumrey (Research Centre for Natural Sciences, Hungary)

O3a-6
9:30-9:45
Properties of nanoparticles affecting simulation of fibrous gas filter performance
Paolo Tronville, R. Rivers (Politecnico di Torino DENERG, Italy)

O3a-7
9:45-10:00
Investigation of the life cycle of titania nps using radiolabeling techniques for highly sensitive np detection
Heike Hildebrand, K. Franke, S. Schymura, A. Freyer, E. Bilz' Reiner Mehnert, E. Mai, C. Isaacson, H. Schug, K. Schirmer, A. Ammann, L. Sigg (Helmholtz-Zentrum Dresden-Rossendorf, Germany)

10:00-10:30
Coffee-break

ROOM B

(Co-Chair: Samir Derrouh)

3b. Characterization

O3b-1
11:15-11:30
CANCELLED
Sonication effects on multi-walled carbon nanotube characteristics for toxicity studies
Soline Allard, S. Che Mansor, M. Ferrie, M. Mayne-L'Hermite, M. Pinault, C. Reynaud (Laboratoire Edifices Nanométriques, DSM-IRAMIS-NIMBE, CEA Saclay, France)

O3b-2
11:30-11:45
Ignition and Explosion Properties of Different Types of Nano-Materials
Arne Krietsch, M. Schmidt , O. Holzschuh, T. Papirer (BAM Federal Institute for Materials Research and Testing, Germany)

O3b-3
11:45-12:00
Characterization of nanoparticle size and state in nanotoxicological and ecotoxicological studies using nanoparticle tracking analysis (nta)
Pierre Peotta, P. Hole, P. Peotta, S. Capracotta, B. Carr (Malvern Instruments, NanoSight, UK/Malvern Instruments, France)

O3b-4
12:00-12:15
Detailed characterization of welding fume in personal exposure samples
Bernadette Quemerais, C. Scott, H. Golshahi (Department of medicine, University of Alberta, Canada)

12:30-13:30 *Lunch*

Thursday 20 November

ROOM C

3c. Instrumentation

O3c-1
14:15-14:30
Hyphenation of nta on-line with af4/mals/icp-ms for the characterisation of nanomaterials in a complex matrix
Dorota Bartczak, H. Goenaga-Infante, P. Vincent (LGC Limited, UK)

O3c-2
14:30-14:45
Towards routine nanoparticle measurements with person-carried instruments
Dirk Dahmann, C. Monz, V. Neumann, C. Asbach, H. Kaminski, A. Maria Todea, C. Möhlmann (Institute for the Research on Hazardous Substances/IUTA, Germany)

O3c-3
14:45-15:00
Design of an exposure chamber for evaluation of personal samplers
Izadi Hossein, B. Quémerais (University of Alberta, Canada)

O3c-4
15:00-15:15
Design of nanoparticle reference materials for sensor development in the context of the eu-project instant
Patrick Knappe, A. F. Thuenemann (BAM Federal Institute for Materials Science and Testing, Germany)

O3c-5
15:15-15:30
Multi-instrument manager tool for data acquisition and data merging of optical and electrical mobility size distributions
Tritscher Torsten, L. Bustin, C. Kykal, O. F. Bischof, E. Filimundi, H.S Han, T. Johnson, S. Elzey, Aaron Avenido (TSI GmbH, Germany)

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

Session 4: Toxicology

Wednesday 19 November

MINATEC AUDITORIUM

(Chair: Günter Oberdörster)

4a. Respiratory tract dosing studies

PL4
8:00-8:35
Nanoparticle Toxicology: A critical appraisal of hazard and risk characterization
Günter Oberdörster (University of Rochester, Department of Environmental Medicine, USA)

O4a-1
8:45-9:00
What is the impact of carbide nanomaterials to the mineral composition of rat lungs? a pixe-µpixe comparative study
Omar Lozano García, S. Lucas, J. Colaux (PMR/NNC/NARILIS/FUNDP, Belgium)

O4a-2
9:00-9:15
The impact of sic and tic nanomaterials in a rat model
Omar Lozano García, S. Lucas, J. Laloy, J.M Dogné, O. Toussaint (PMR, Belgium)

O4a-3
9:15-9:30
Organ weight changes in mice after long-term inhalation exposure to manganese oxides nanoparticles
Tomá Zeman, M. Buchtová, I. Míšek, O. Šerý, B. Dočekal (University of Defence, Department of Population Protection, Czech Republic)

O4a-4
9:30-9:45
Silica nanoparticles cause pleural effusion, pericardial effusion and pulmonary fibrosis in rats
Song Yuguo, X. Zhu, W. Cao, Y. Niu, and L. Si (Capital University of Medical Sciences, China)

4b. Carbon based nano materials, in vitro and in vivo studies

O4b-1
9:45-10:00
Interlab study on nanotoxicology of representative graphene oxide
Nelson Duran, D. S.T. Martinez, G. Z. Justo, R. de Lima, V. Lúcia de Castro, G. A. Umbuzeiro, E. Barbieri, M. Durán, P. S. Melo, O.L. Alves, W. J. Fávaro (Universidade Estadual de Campinas–UNICAMP/NanoBioss, Brazil)

10:00-10:30 *Coffee-break*

ROOM C

O4b-2
11:15-11:30
Biological response to purification and acid functionalization of carbon nanotubes
Agathe Figarol, J. Pourchez, D. Boudard, V. Forest, J.M Tulliani, J.P Lecompte, D. Bernache-Assolant, M. Cottier, and P. Grosseau (Ecole Nationale Supérieure des Mines, SPIN-EMSE, CNRS, France)

O4b-3
11:30-11:45
Cytotoxicity evaluations of carbon dots with different surface charge
Marketa Havrdova, K. Hola, J. Skopalik, K. Tomankova, O. Kozak, M. Petr, K. Cepe, K. Polakova, A.B. Bourlinos, E.P. Giannelisd J.Tucek, R. Zboril (Regional Centre of Advanced Technologies and Materials, Czech Republic)

O4b-4
11:45-12:00

Interaction of nanomaterials with toll like receptor ligands: characterization of the biomolecule corona and influence on the cellular response
Isabella Radauer-Preiml, A. Andosch, M. Himly, M. S. P. Boyles, J. Horejs-Hoeck, U. Luetz-Meindl, C. Huber, A. Duschl (Department of Molecular Biology, Division of Allergy and Immunology, University of Salzburg, Austria)

4c. Cell-free in vitro testing

O4c-1
12:00-12:15

Characterization of nanoparticulate surfaces and their relation to different protein coronae
Doris Segets, W. Lin, U. Weichsel, J. Walter, W. Peukert, M. Pink, S. Schmitz-Spanke (Institute of Particle Technology, Germany)

O4c-2
12:15-12:30

Computational methods for the toxicological assessment of manufactured nanomaterials
Lara Lamon, D. Asturiol, K. Gerloff, T. Palosaari, J. Bessems, K. Aschberger, A. Worth (European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Italy)

12:30-13:30 *Lunch*

ROOM C

(Co-Chair: Marie Carriere)

O4c-3
13:30-13:45

Assessment of the oxidative potential of nanoparticles: comparison and improvement of methods
Mathilde Delaval, W. Wohlleben, L. Ma-Hock, R. Landsiedel, A. Baeza-Squiban, S. Boland (Univ Paris Diderot, France)

O4c-4
13:45-14:00

Nanomaterials solubility/biodurability and reactivity in synthetic biological fluids and cell media
Jensen Keld Alstrup (The National Research Centre for the Working Environment, Denmark)

O4c-5
14:00-14:15

Easy to digest? A comprehensive in vitro approach to monitor the fate of orally ingested nanoparticles
Patrick Knappe, A. F. Thuenemann (BAM Federal Institute for Materials Science and Testing, Germany)

4d. Correlating cell, cell-free and in vitro studies

O4d-1
14:15-14:30

Study of lung lining fluid interactions with nanoparticles: Towards more relevant in vitro toxicity tests
Fanny Mousseau, E. Seyrek, J.F. Berret (Université Paris Diderot, Laboratoire MSC, France)

O4d-2
14:30-14:45

Toxicity of Ag nanoparticle and induction of an inflammatory response in the GI tract and mediation of this toxicity by associated bio-fluid components
Anna Murphy, K. Sheehy, A. Casey, G. Chambers (Nanolab Research Centre, Focas Institute, Dublin Institute of Technology, Ireland)

O4d-3
14:45-15:00
Cerium dioxide nanoparticles affect in vitro fertilization in mice
Lise Preaubert, B. Courbiere, V. Achard, V. Tassistro, T. Orsiere, J.Y. Bottero, J. Rose, M. Auffan, J. Perrin (Aix-Marseille Université, Biogénotoxicologie, Faculté de Médecine, France)

O4d-4
15:00-15:15
Treatment of cells with nanoparticles boosts intercellular communication
Julia Schoelermann, T. Sprenger, M. Roxana Cimpan (Department for Clinical Dentistry, University of Bergen, Norway)

O4d-5
15:15-15:30
Titanium dioxide nanoparticles toxicology: towards more physiological in vitro exposure models?
Lucie Armand, M. Biola-Clier, M. Dilger, S. Muelhopt, C. Schlager, V. Collin-Faure, H.R Paur, S. Diabate, C. Weiss, N. Herlin-Boime, T. Rabilloud, M. Carriere (Laboratoire Lésions des acides nucléiques, UMR E3 CEA, UJF, DSM, INAC, SCIB, CEA, France)

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

Thursday 20 November

S225

(Co-Chair: Claude Emond)

O4d-6
10:30-10:45
An approach to evaluate which in vitro model and exposure method is more predictive for in vivo biological responses
Thomas Loret, E. Peyret, T. Amodeo, M. Hanot-Roy, B. Trouiller, C. Egles, G. Lacroix (INERIS, France / Université de Technologie de Compiègne, France)

4e. Cellular uptake, dosimetry and distribution and oxidative potential

O4e-1
10:45-11:00
How do oxide and carbide enm dispersions evolve in an in vitro assessment?
Omar Lozano García, J. Mejia, J.P. Piret, C. Saout, C. C. Zouboulis, O. Toussaint, S. Lucas (Research Centre for the Physics of Matter and Radiation, PMR, Namur Nanosafety Centre, NNC, Namur Research Institute for Life Sciences, NARILIS, University of Namur, Belgium)

O4e-2
11:00-11:15
Characterization of the oxidative potential of nanomaterials
Leticia A Santiago, L. Chi Bui, J. Dairou, S. Boland, A. Baeza-Squiban (University Paris Diderot, BFA, UMR CNRS, France)

O4e-3
11:15-11:30
Fate of metallic nanoparticles in cellular models: dissolution, speciation and complexation in cellulo probed by synchrotron-based techniques
Giulia Veronesi, E. Brun, T. Gallon, M. Cuillel, P. Charbonnier, F. Rollin-Genetet, C. Vidaud, P. Delangle, C. Aude-Garcia, T. Rabilloud, E. Mintz, I. Michaud-Soret, M. Carrière (CEA/CNRS, LCBM/Univ. Grenoble Alpes, France)

O4e-4
11:30-11:45
The influence of size, time and dose on the translocation of gold nanoparticles across mouse and human alveolar epithelial cell monolayers
Gerald Bachler, N. Von Goetz, K. Hungerbuhler, Y. Umehara, L. Rodriguez-Lorenzo, A. Petri-Fink, B. Rothen-Rutishauser, S. Losert (ETH Zürich - Institute for Chemical and Bioengineering, Switzerland)

O4e-5
11:45-12:00
Comparison of in vitro cytotoxicity and oxidative stress of Poly (propylene imine) and poly (amido amine) dendritic nanoparticles
Khalid Humza, L. O'Neill, H. J. Byrne Mark Byrne, A. Heise, S. Prasanna Mukherjee (Focas Research Institute, Ireland)

4f. Brain and skin as targets and biokinetic modeling

O4f-1
12:00-12:15
The need for physiologically-based models to predict nanoparticle biodistribution
Gunnar Johanson, U. Carlander, C. Emond, (Institute of Environmental Medicine,Sweden Karolinska Institutet, BioSimulation Consulting Inc, Sweden)

O4f-2
12:15-12:30
Effects on the nervous system of exposure to engineered nanomaterials – an overview
Mattsson Mats-Olof, M. Simkó (Austrian Insitute of Technology, Austria)

12:30-13:30 *Lunch*

O4f-3
13:30-13:45
Cyto- and genotoxicity of silver nanoparticles in human keratinocytes: infulence of surface coating
Helena Oliveira, V. Bastos, T. Pedrosa, J. Miguel P Ferreira de Oliveira, C. Santos, L. Duarte (CESAM & Laboratory of Biotechnology and Cytomics, Portugal)

O4f-4
13:45-14:00
Nano-TiO2 modulates the dermal sensitization potency of DNCB after topical exposure
Peter Hoet, Stijn Smulders, J. Vanoirbeek (KU Leuven, Faculty of Medicine, Department of Public Health and Primary Care, Belgium)

Session 5: Environmental interactions of nanomaterials

Wednesday 19 November

MINATEC AUDITORIUM

(Chair: Jean Yves Bottero)

PL5 10:30-11:05
Nanotechnology, global development in the frame of environmental risk forcasting. a necessity of interdisciplinary research
Jean Yves Bottero, M. Auffan, D. Borschnek, P. Chaurand, J. Labille, C. Levard, A. Masion, J. Rose, M.R Wiesner (CEREGE, France)

O5a-1 11:15-11:30
Imogolites as a model for the study of nanoparticle's ecotoxicity toward Pseudomonas brassicacearum
Astrid Avellan, C. Levard, J. Rose, A. Masion, W. Achouak (CEREGE, CNRS, France)

O5a-2 11:30-11:45
Relationships between nano-design of ceria nanoparticle and their impact on a terrestrial soil-microbe-plant ecosystem
Mohamed Hamidat, C. Simonet, M. Barakat, P. Ortet, J. Rose, W. Achouak, C. Santaella (Lab Ecologie Microbienne de la Rhizosphère et Environnements Extrêmes, CNRS-CEA, France)

O5a-3 11:45-12:00
Environmental transformations of silver nanoparticles: impacts on stability, bioavailability and toxicity
Clément Levard, R. Ma, J. Stegemeier, G.V. Lowry, S. Mitra, F.M. Michel, N. Bossa, J. Rose and G.E Brown (CNRS, Aix-Marseille University, IRD, CEREGE, France), (Surface and Aqueous Geochemistry Group, Department of Geological & Environmental Sciences, Stanford University, USA), (Department of Civil and Environmental Engineering, Carnegie Mellon University, United States), (CEINT)

O5a-4 12:00-12:15
Comparison of tio2 nano-objects toxicity on caenorhabditis elegans
Gladys Saez, Q. Le Trequesser, G. Devès, P. Barberet, C. Michelet, M. Petrel, E. Gontier, D. Dupuy, M.H. Delville, H. Seznec (Université de Bordeaux, Centre Etudes Nucléaires de Bordeaux Gradignan, France)

12:30-13:30 *Lunch*

ROOM B

(Chair: Jean Yves Bottero)

O5a-5 14:15-14:30
Nanoparticles interactions with plants from model to ecosystem: nano-design matters
Catherine Santaella, M. Hamidat, C. Simonet, M. Barakat, P. Ortet, W. Achouak (Lab Ecologie Microbienne de la Rhizosphère et Environnements Extrêmes, CNRS-CEA, France), (iCEINT, France)

O5a-6 14:30-14:45
Environmental Mobility of Carbon Nanotubes
Stefan Schymura, J. Kulenkampff, K. Franke, J. Lippmann-Pipke (HZDR, Institute of Resource Ecology, Germany)

O5b-1
14:45-15:00
Evaluation of the effects of nitric oxide-releasing nanoparticles on plants
A. B. Seabra, Anderson E.S. Pereira, A. M. Narciso, L. F. Fraceto (Universidade Estadual de Campinas, Campus Universitário Zeferino Vaz, Brazil)

O5b-2
15:00-15:15
Effect of silver nanoparticles on estuarine bivalves scrobicularia plana
Carole Bertrand, L. Poirier, A. Zalouk-Vergnoux, S. Devin, M. Auffan, M. Tella, J. Labille, H. Perrein-Ettajani, L. Giamberini, C. Mouneyrac (Université de Lorraine/LIEC, France)

05b-3
15:15-15:30
Comparative study of the two types of nanoparticles on fresh water microcosm at low level concentrations
Kumar Deepak, N. Chandrasekaran, A. Mukherjee (VIT University, Centre for Nanobiotechnology, India)

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

ROOM B

(Co-Chair: Jérome Rose)

O5b-4
16:00-16:15
Comprehensive study on the impact of tio2 nps on biofilm formation of the freshwater sediment bacterial isolates and their consortium: projected risks for aquatic environment
Kumari Jyoti, N. Chandrasekaran, A. Mukherjee, R. Nagarajan (Centre for Nanobiotechnology, VIT University, India)

O5b-5
16:15-16:30
Aging of nano-products and impacts toward aquatic organisms across a salinity gradient
Marie Tella, E. Mohr, A. Pariat, D. Borschneck, B. Angeletti, M. Cabie, J.H. Ferrasse, A. Masion, C. Mouneyrac, L. Giamberini, M. Auffan (CNRS, Aix-Marseille Université, CEREGE, France), (GDRi iCEINT, France)

O5c-1
16:30-16:45
Genotoxic and cytotoxic effects of silver nanoparticles in the bivalve scrobicularia plana
Amélie Châtel, P.E. Buffet, H. Perrein-Ettajani, I. Métais, A. Zalouk-Vergnoux, L. Poirier, D. Gilliland, C. Risso-de Faverney, M. Guibbolini, E. Valsami-Jones, C. Mouneyrac (LUNAM Université/Université Catholique de l'Ouest, France)

O5c-2
16:45-17:00
Chronic contamination of aquatic mesocosms by ag nanoparticles with different shape
Marie Tella, M. Auffan, C. Levard, A. Thiéry, C. Santaella, L. Brousset, C. Pailles, J. Issartel, E. Mohr, W. Achouak, B. Angeletti, J. Rose, M. R. Wiesner, J.Y. Bottero (CNRS, Aix-Marseille Université, CEREGE, France), (GDRi iCEINT, France)

Session 6: Nanomaterials Release

Wednesday 19 November

MINATEC AUDITORIUM

(Chair: Wendel Wohlleben)

PL6
13:30-14:05 Nanotechnology commercialization and the need of release testing along the product lifecycle
Wendel Wohlleben (BASF, Germany)

6.a. Release by Mechanical Stress

O6a-1
14:15-14:30 Characterization methods for MWCNT polymer composites and nanocomposite release particles
Keana Scott *(Invited Speaker)*, J. Woodcock, C. Davis, J. Gilman, G. Myers, J. Schumacher, A. Meyers (National Institute of Standards and Technology, USA)

O6a-2
14:30-14:45 An overview of release from solid nanocomposites
Stephan J. Froggett, Froggett & Associates (LLC, Seattle, WA)

O6a-3
14:45-15:00 Nanoaerosol release characteristics of silver nanocomposite by sanding test
Gwi-Nam Bae *(Invited Speaker)*, K.S. Kim, J.H. Ji, D.Woo, J.B. Kim, J.H. Kim, H.J. Lee (Korea Institute of Science and Technology, Korea)

O6a-4
15:00-15:15 Measurement of nanoparticles release during drilling of polymer nanocomposites
Laura Gendre, K. Blackburn, V. Marchante Rodriguez, J. Brighton and H. Abhyankar (Cranfield University, Centre for Automotive Technology, UK)

O6a-5
15:15-15:30 Particle release from single-wall and multiwall carbon nanotubes in polystyrene-based composites during grinding
Ogura Isamu, M. Shigeta, M. Kotake, M. Uejima, K. Honda (National Institute of Advanced Industrial Science and Technology, Japan), (Technology Research Association for Single Wall Carbon Nanotubes, Japan)

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

ROOM C

O6a-6
16:00-16:15 Towards harmonized investigations on the release of nanomaterials from composites during mechanical treatment – results from an interlaboratory comparison
C. Asbach, J. Meyer, S. Clavaguera, B. Fiorentino, H. Kaminski, S. Kreckel, M.W. Meier, B. Stahlmecke, W. Wohlleben and T.A.J. Kuhlbusch (Institut für Energie - und Umwelttechnik, Germany), (Univ. Grenoble Alpes, PNS, CEA, France), (BASF, Germany)

6b. Release by Washing/Leaching, Fire, End-of-Life

(Chair: Jean-Francois Damlencourt)

O6b-1 16:15-16:30
Nanomaterials release from comercial fabrics for sportswear and automotive applications
Elisabet Fernández-Rosas, Socorro Vázquez-Campos *(Invited Speaker)*, A. Vílchez, V. Pomar, D. González-Gálvez, M. Blázquez, A. Satti (LEITAT Technological Center, Spain)

O6b-2 16:30-16:45
Tracking nanomaterials through the laundry wash cycle: release, dissolution and complexation
Denise M. Mitrano and B. Nowack (EMPA – Swiss Federal Laboratories for Materials Science and Technology, Switzerland)

O6b-3 16:45-17:00
Effects of released particles: nanohouse results
Peter Hoet (Pneumology, Leuven)

O6b-4 17:00-17:15
Leaching potential of nanomaterials during different human contact scenarios and end-of-life
Steffen Foss Hansen, A. Mackevica, L. Heggelund, M. Emil Olsson, A. Boldrin (Department of Environmental Engineering, Technical University of Denmark, Denmark)

O6b-5 17:15-17:30
What is emitted from combustion of nanocomposites? results on pu and pe polymers with carbon black, nanotubes, iron oxides, organic pigments
Georgios A. Sotiriou, D. Singh, Wendel Wohlleben, and P. Demokritou (Center for Nanotechnology and Nanotoxicology, U.S.A)

O6b-6 17:30-17:45
Characterising the release of carbon nanotubes from burning cnt-polymer nanocomposite
Antonis Christou and A. A. Stec (Centre for Fire and Hazard Sciences, University of Central Lancashire, UK)

20:00-23:30 *Cocktail Party-Château de Sassenage*

Thursday 20 November

ROOM B

6c. Release by Weathering

(Chair: Tinh Nguyen)

O6c-1
08:45-09:00
Development of a conceptual framework for evaluation of nanomaterials release from nanocomposites: environmental and toxicological implications
Alexander Orlov *(Invited Speaker)*, J. Ging, R. Tejerina-Anton, G. Ramakrishnan, M. Nielsen, K. Murphy, JM. Gorham, T. Nguyen (Materials Science and Engineering, Stony Brook University, USA)

O6c-2
9:00-9:15
Mechanisms of entangled cnt layer formation and its resistance to release during uv irradiation of polymer nanocomposites
Tinh Nguyen and L. Sung (Scientific Consulting, National Institute of Standard and Technology, USA)

O6c-3
9:15-9:30
Lifecycle of commercial photocatalytic nanocoatings: nanoparticles aerosol emission during mechanical and environmental stresses application
Neeraj Shandilya, O. Le Bihan, C. Bressot, M. Morgeneyer (INERIS, France)

O6c-4
9:30-9:45
In search of factors affecting the release of nanomaterial from product's life cycle: the guidenano project
Alexandro Vílchez Villalba, Stefano Zuin, A. Massari, S. Vázquez-Campos, D. Boutry (LEITAT Technological Center, Spain), (Venice Research Consortium, Italy)

O6c-5
9:45-10:00
Insight into mechanisms leading to the release of ceo_2 nanoparticles embedded in an acrylic wood coating
Lorette Scifo, P. Chaurand, A. Avellan, N. Bossa, A. Masion, M. Auffan, D. Borschneck, J. Labille, J.Y. Bottero and J. Rose (Aix-Marseille Université, CNRS, IRD, CEREGE UMR 7330, France), (Tecnalia-France, France)

O6c-6
10:00-10:15
Investigation of the nanoparticles release mechanism from paints due to environmental and mechanical aging
Brice Fiorentino, D. Boutry, J.F. Damlencourt (Univ. Grenoble Alpes, PNS, CEA, France)

Session 7: Industrial production and prevention

Thursday 20 November

MINATEC AUDITORIUM

(Chair: Eric Gaffet)

7a. Safer by design approach

PL7
8:00-8:35
Nanomaterials : Industrial Production and Prevention
E. Gaffet (Institut Jean Lamour, UMR 7198 CNRS – Université de Lorraine, France)

O7a-1
8:45-9:00
Dustiness testing: a support to nanosafety by design
Olivier Le Bihan, C. Bressot, C. Dutouquet, T. Jayabalan Yuri Fedutik, A. Antipov (PlasmaChem GmbH, Germany), (INERIS, France)

O7a-2
9:00-9:15
Wet state characterization as key step in a safety by design approach
Camilla Delpivo, S. Ortelli, M. Blosi, A. Vaccari, T. Syed, A. L. Costa (Nanotechnologies and Colloidal Processing, CNR-ISTEC, Italy)

O7a-3
9:15-9:30
Safety by design to control the biological reactivity of nanosilver
Magda Blosi, S. Ortelli, C. Delpivo, D. Gardini, M. G. Bianchi, M. Allegri, O. Bussolati, E. Bergamaschi, A. Luisa Costa (CNR-ISTEC, Institute of Science and Technology for Ceramics, National Research Council, Italy)

O7a-4
9:30-9:45
Nano cuo case study: integration of safety by molecular design approach
Anna.L. Costa, L. Viale, M. Blosi, S. Ortelli (ISTEC – CNR, Italy)

10:00-10:30 *Coffee-break*

7b. Safe equipment, collective and individual protection

ROOM B

(Co-chair: Catherine Durand)

O7b-1 11:15-11:30 Efficiency of current alternatives for personal dermal protection towards nanohydrosols
Delphine Boutry, J.F. Damlencourt (Univ. Grenoble Alpes, PNS, CEA, France)

O7b-2 11:30-11:45 Secured nanomaterial workplaces at the liten-pns (CEA Grenoble - Nano Safety Platform) open to industrials, as practical case
Catherine Durand, A. Sperandio, D. Boutry, J.F. Damlencourt, V. Fenneteau, C. Tardif (Univ. Grenoble Alpes, PNS, CEA, France)

O7b-3 11:45-12:00 Nanosecured plateform to assess risks along the industrial lifecycle of nanomaterials
Bruno Debray, A. Vignes, J. Bouillard (INERIS, France)

7c. Static and dynamic containment

O7c-1 12:00-12:15 Toward understanding the mechanisms and the kinetic of nanoparticle penetration through protective gloves
Ludwig Vinches, M. Zemzem, N. Boutrigue, S. Hallé Kevin, J. Wilkinson, C. Peyrot, L. Lemarchand, N. Tufenkji (École de technologie supérieurs, Canada)

12:15-13:30 *Lunch*

8: Life Cycle Analysis

Thursday 20 November

MINATEC AUDITORIUM

(Chair: Bernd Nowack)

PL8
10:30-11:05
The life cycle perspective as basis for assessing environmental risks of engineered nanomaterials
Bernd Nowack (EMPA, Swiss Federal Laboratories for Materials Science and Technology, Switzerland)

O8a-1
11:15-11:30
Nanomaterials in construction and demolition – how can we assess the risk if we don't know where they are?
Wendy Jones, A. Gibb, C. Goodier, P. Bust, M. Song (School of Civil and Building Engineering, Loughborough University, UK)

O8a-2
11:30-11:45
Nanomaterials in construction and demolition waste in switzerland
Ingrid Hincapié, A. Caballero, B. Nowack (EMPA – Swiss Federal Laboratories for Material Science and Technology, Switzerland)

O8a-3
11:45-12:00
Flows of engineered nanomaterials through the recycling process in Switzerland
Alejandro Caballero Guzman, T. Sun, B. Nowack (EMPA, Swiss Federal Laboratories for Materials Science and Technology, Switzerland)

O8a-4
12:00-12:15
Environmental impacts of multiwalled carbon nanotubes (mwcnt) and platinum in fuel cell technology
Dominic Notter, K. Kouravelou, N. Tudela Haberland, (Department of Mobility, Energy and Environment, Swiss Federal Laboratories for Materials Science and Technology, Switzerland)

O8a-5
12:15-12:30
Life cycle based socio-economic assessment combining environmental impact, occupational health risks and health benefits for nanosilver coated door handles
Tom Ligthart, H.Buist, E. Kuijpers, W. Fransman, M. de Weerd (TNO - Climate Air and Sustainability, Netherlands)

12:30-13:30 *Lunch*

ROOM B

(Chair: Bernd Nowack)

O8a-6
14:15-14:30
Lca-integrated human health risk assessment: application in four case studies on enm
Wouter Fransman, H. Buist, E. Kuijpers, E. Zondervan, D. H Brouwer (TNO, Netherlands)

O8a-7
14:30-14:45
Licara nanoscan: evaluating benefits and risks over the life cycle of nanoproducts
Esther Zondervan, D. Brouwer, T. Van Harmelen, D. Notter, R. Hischier, C. Som (Netherlands Organisation for Applied Scientific Research-TNO, Netherlands)

O8a-8
14:45-15:00
Freshwater ecotoxicity characterisation factor for engineered nanoparticles - the case study of nano-titaniumdioxide
Beatrice Salieri, R. Hischier, S. Righi, A. Pasteris, S. Irving Olsen (Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland)

O8a-9 Framework for human health characterization factor calculation of tio2 nanoparticles

15:00-15:15 Martina Pini, A. Maria Ferrari, B. Salieri, R. Hischier, B. Nowack (Department of Engineering Sciences and Methods, University of Modena and Reggio Emilia, Italy)

O8a-10 Toxicity characterization factors for nanomaterials: current developments and limitations

15:15-15:30 Gonzalo Rodriguez-Garcia, B. Zimmermann, M. Baumann, M. Weil (Helmholtz-Institute Ulm for Electrochemical Energy Storage, Germany)

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

Session 9: Regulation and standardization

Thursday 20 November

PETIT SALON

(Chair: Daniel Bernard)

PL9 13:30-13:45 Regulation Perspectives
Daniel Bernard (CEA, Senior Scientific Advisor, NanoSafety Platform, Grenoble, France)

O9a-1 13:45-14:00 Nanomaterials in food – current and future applications and regulatory aspects
Karin Aschberger, S. Gottardo, V. Amenta, M. Arena, H. Bouwmeester, P. Brandhoff, H. Rauscher, R. Schoonjans, M. Vittoria Vettori, R. Peters (IHCP-Nanobiosciences, JRC Ispra, Italy)

O9a-2 14:00-14:15 Classification and Reporting of Nanostructured Silica Materials
Atluri Rambabu, K. A. Jensen (National Research Centre for the Working Environment NRCWE, Denmark)

O9a-3 14:15-14:30 International and European standardization in nanotechnology; How standardization can help industry and regulators in developing safe products?
Jean-Marc Aublant (LNE, France)

O9a-4 14:30-14:45 National nano registers: admissibility under EU law and regulatory uncertainties
Anthony Bochon (Squire Patton Boggs LLP, Brussels)

O9a-5 14:45-15:00 Paving the way from research to standards in the field of nanotechnologies: the nanoSTAIR support and pre-normative work
Benoît Hazebrouck, O. Salvi, B. Caillard, with the participation of QualityNano (EU-VRi European Virtual Institute for Integrated Risk Management. Haus der Wirtschaft, Germany)

O9a-6 15:00-15:15 The nanostair strategy: a new strategic proposal to impulse standardization in nanotechnology research
Jesús Lopez de Ipiña, O. Salvi, B. Hazebrouck, A. Jovanovic, F. Carre, A. Saamanen, D. Brouwer, M. Schmitt, S. Martin (TECNALIA, Spain)

15:15-15:30 Debate Regulation

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

Session 10: Commercial equipment

(Chair: Raphael de Thoury)

Wednesday 19 November

MINATEC AUDITORIUM

O10a-1 Nanosafety platform
16:00-16:15 Fréderic Amblard (Univ. Grenoble Alpes, PNS, CEA, France)

O10a-2 A software infrastructure dedicated to nanosafety
16:15-16:30 Johann Foucher, N. Feltin, A. Delvallée, S. Ducourtieux, F. Piquemal, J.P. Lecailliez (POLLEN Technology, France)

O10a-3 Mini particule sampler for nanosafety
16:30-16:45 Cédric NEVEU (ECOMESURE, France)

O10a-4 Miniature nanoparticle sensors for exposure measurement and tem sampling
16:45-17:00 Martin Fierz, D. Meier, P. Steigmeier and H. Burtscher (Naneos particle solutions gmbh, Switzerland), (University of applied sciences northwestern Switzerland, Switzerland)

O10a-5 NanoBadge
17:00-17:15 Raphel de Thoury (NanoBadge, Alcen)

O10a-6 Nano-scale Optical and Hyperspectral Microscopy
17:15-17:30 Sam Lawrence, Nicolas Gonzalez (CytoViva, Inc, USA ,Schaefer Techniques, Centre d'activité, France)

20:00-23:30 Cocktail Party-Château de Sassenage

Session 11: Risk Management

Thursday 20 November

MINATEC AUDITORIUM

(Chair: Olivier Salvi)

PL11 13:30-14:15
Risk Management for nanomaterials: what are the existing methods and tools?
Olivier Salvi, E. Frejafon, A. Jovanovic, M. Löscher, B. Hazebrouckh (European Virtual Institute for Integrated Risk Management, France)

O11a-1 14:15-14:30
Application of risk assessment approaches on pilot scale process lines using nanomaterials within the SANOWORK project
Jayabalan Thangavelu, A. Janes, B. Debray, G. Fayet (INERIS, France)

O11a-2 14:30-14:45
Strategies, methods and tools for managing nano-risks in construction
Jesús Lopez de Ipiña, C. Vaquero, D. Boutry, M. Pilou, P. Neofytou, E. Jankowska, R. Pina, I.Larraza, S. Fernández, K. Otkallo, A. Pintea, C.Salazar, B. Hargreaves, R. Ciobanu, B. Hazebrouck, H. Stockmann-Juvala, V.Vaananen, D.Y. H. Pui, D. Thompson (TECNALIA, Spain)

O11a-3 14:45-15:00
Nanomaterials at the construction sector – tools and guidelines for occupational health care units
Säämänen Arto, V. Väänänen, T. Kanerva, A.K Viitanen, S. Uuksulainen, H. Stockmann-Juvala (Finnish Institute of Occupational Health, Uimalankatu, Finland)

O11a-4 15:00-15:15
Risk assessment of nanocarbons: use the analytical hierachy process and control banding aproach on safety management of carbon nanomaterials
Lenz e Silva Guilherme, R.Hurt (University of São Paulo – Dept. of Metallurgy & Materials Engineering, Brazil), (Brown University – School of Engineering, Institute for Molecular and Nanoscale Innovation, USA)

O11a-5 15:15-15:30
Field campains of measurement of nanoaerosols: from synthesis of the results to an EHS prevention tool
Catherine Durand, E. Zimmermann, S. Artous, D. Locatelli, P. Nobile, S. Derrough, B.Belleville (Univ. Grenoble Alpes, PNS, CEA, France)

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

MINATEC AUDITORIUM

(Co-chair: Olivier Wistchger)

O11a-6 16:00-16:15
A standardized non-instrumental method for tracking workstations concerned with exposure to nano-objects and their aggregates and agglomerates in companies dealing with engineering
Irina Guseva Canu, S. Ducamp, L. Delabre, S. Audignon-Durand, C. Ducros, C. Durand, Y. Iwatsubo, D. Jezewski-Serra, O. Le Bihan, S. Malard, A. Radauceanu, M. Reynier, M. Ricaud, and O. Witschger (French Institute for Health Surveillance, InVS, France)

O11a-7 16:15-16:30
Risk assessment in a research laboratory during sol-gel synthesis of nano-tio2
Francisco Silva, P. Arezes, P. Swuste (Technological Centre for Ceramic and Glass, Portugal), (University of Minho, Portugal), (Delft University of Technology, Nederlands)

O11a-8 16:30-16:45
Announcement on hazardous substances 527 - Manufactured Nanomaterials
Johannes Pelzer, C. Schumacher (Institute of Occupational Safety and Health of the German Social Accident Insurance, IFA, Germany)

O11a-9 16:45-17:00
Qualitative risk assessment during polymer mortar test specimens preparation – methods comparison
Silva Francisco, P. Arezes, P. Swuste, S.P.B. Sousa, M.C.S. Ribeiro, J.S. Baptista (Technological Centre for Ceramic and Glass, Portugal), (School of Engineering, University of Minho, Portugal)

17:00 End of the Conference - Conclusion

Session 12: Nanoresponsible Development

Tuesday 18 November

ROOM C

(Chair: Pieter van Broekhuizen)

O12a-1
14:00-14:20
Stakeholder engagement in nanotechnologies. Dialogue and outreach for responsible research & innovation in nanotechnologies
Pieter Van broekhuizen, H. Krop, A. Farchi (IVAM UvA, Netherlands)

O12a-2
14:20-14:40
Nanoresponsible development: framing a model of innovation market uptake of nano-enabled products
Mariia Ostapchuk, C. Auplat, P. Boucard, J. M. Brignon (PSL, Université Paris-Dauphine, France), (Novancia Business School Paris, France), (INERIS,France)

O12a-3
14:40-15:00
Licara - guideline towards sustainable competitiveness of nanoproducts
Claudia Som, E. Zondervan-van den Beuken, T. van Harmelen, R. Hischier, B. Nowack, I.Hincapie, H. E. Buist, W. Fransman, J. Güttinger (Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland), (TNO, Netherlands), (NANO-CLUSTER BODENSEE, NCB, Switzerland)

O12a-4
15:00-15:20
The ne3ls network's quadruple helix model of innovation towards a responsible development of nanotechnology
Charles-Anica Endo, M.H. Parizeau, C. Emond, C. Beaudry (École de technologie supérieure Montréal, Canada)

O12a-5
15:20-15:40
Socio-economic analysis of a nano-enabled technology: nano-tio2 coatings solar panel efficiency
Pierre Boucard, J.M. Brignon (National Institute for Industrial Environment and Risks, France)

O12a-6
15:40-16:00
Areas of discussion about work, resources for prevention of risks related to nanomaterials
Catherine L'Allain, S. Caroly, E. Drais (Laboratoire LIP, Université de Grenoble INPG, France)

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

16:30-18:30 Debate

Panel-discussions

Tuesday 18 November

ROOM C

(Moderator: Pieter van Broekhuizen)

16:30-17:30 **Responsible development: how to do with nano? Comparison between the application of the principle of precaution in nanomaterials, and the other emerging risks.(Moderator:**
Pieter van Broekhuizen (IVAM UvA, Netherlands), C. Auplat (Novancia Business School Paris, France), J. M. Brignon (PSL, Université Paris-Dauphine, France), Charles-Anica Endo (Ecole de technologie supérieure Montréal, Canada), Claudia Som (Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland), Catherine L'Allain (Laboratoire LIP, Université de Grenoble INPG, France)

(Moderator: Jérôme Rose)

17:30-18:30 **Nanomaterials: risks and benefits for the environment.**
Jérôme Rose (CEREGE, France), Bernd Nowack (EMPA, Switzerland), Wendel Wohlleben (BASF, Germany), Mark Wiesnier (Duke University, CEINT, USA), Derk Brouwer (TNO, Netherlands)

Wednesday 19 November

ROOM B

(Moderator: Claude Emond)

17:00-18:00 **Nanoparticle of Reference or the Reference Protocol: which ones do we need to embrace for a significant progress in Health Risk Assessment? Pros and Cons. (*)**
Claude Emond (BioSimulation Consulting Inc., Newark, DE, USA), Gunnar Johanson (Institute of Environmental Medicine, Karolinska Institutet, Sweden), Olivier Witschger (Aerosol Metrology Laboratory INRS, France), Peter Hoet (Department of Environmental and Insurance Medicine, Katholieke Universiteit Leuven, Belgium), Gunter Oberdorster (School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY, US)

Thursday 20 November

PETIT SALON

(Moderator: Daniel Bernard)

15:20-15:40 **Regulation and Standardization**
Moderator: Daniel Bernard (CEA, France), Karin Aschberger (IHCP-Nanobiosciences, Italy), Rambabu Atluri (National Research Centre for the Working Environment, Denmark), Jean-Marc Aublant (LNE, France), Anthony Bochon (Squire Patton Boggs LLP, Brussels), Benoît Hazebrouck (EU-VRi European Virtual Institute for Integrated Risk Management, Germany), Jesús López de Ipiña (TECNALIA, Spain)

(*) P9.4 Reference nanoparticles or reference protocol: what should come first to make significant progress in health risk assessment?

Satellite meetings and workshops

Tuesday 18 November

PETIT SALON

18:30-21:00 **HARMONIZATION MEASUREMENT STRATEGY GROUP MEETING**
5tht Workshop Harmonization Strategy
Open workshop.
Coordinator: Derk H. Brouwer (TNO, NL)

Wednesday 19 November

PETIT SALON

8:00-15:30 **SCAFFOLD**
Closed Workshop
SCAFFOLD is an industrial oriented idea specifically addressed to provide practical, robust, easy-to-use and cost effective solutions for the European construction industry, regarding current uncertainties about occupational exposure to MNMs
Coordinator: Jesús M. Lopez de Ipiña (Fundacíon TECNALIA, Spain)

13:30-18:00 **MARINA- MANAGING RISKS OF NANOMATERIALS**
Open meeting
The MARINA project is a major new European Commission Framework 7 project to develop reference methods for managing the risk of engineered nanoparticles and engineered nanomaterials (ENM).
The aim of MARINA is to develop and validate the Risk Management Methods for Nanomaterials.
Coordinator: Dr Lang Tran (Institute of Occupational Medicine, UK)

Thursday 20 November

PETIT SALON

8:30 12:30 **CHARACTERIZATION GROUP MEETING**
Contact: Chantal Tardif (CEA, PNS, France)

ROOM C

8:30-12:30 **NANODIODE**
Open meeting

NanoDiode establishes an innovative, coordinated program for outreach and
NanoDiode, launched in July 2013 for a period of three years, establishes an innovative, coordinated program for outreach and dialogue throughout Europe so as to support the effective governance of nanotechnologies. The project integrates vital engagement activities along the innovation value chain, at the levels of research policy, research & development (R&D), and the use of nanotechnological innovations throughout society.
Coordinator : Pieter van Broekhuizen, IVAM, Netherlands

Friday 21 November

B2/270
CEA

9:00-15.00 **NANOINDEX**

Closed Workshop.

Exposure to airborne manufactured nanomaterials (MNMs) can best be assessed by measuring the individual exposure in the breathing zone of an individual. The project NanoIndEx will determine personal exposure to MNMs and thoroughly investigate the possibilities of personal monitors and samplers.
Coordinator : Christof Asbach (IUTA, Germany)

Poster Session

Tuesday 18 November

GRAND SALON

Poster Session: 18:00-21:00

P1-1 Single step synthesis of graphene oxide using agricultural sugarcane waste materials
Thirunavukkarasu Somanathan, K. Prasad, A. Sarvanan, V. Mohanakrishna, N. Kiruthika, A. Abilarasu and M. Shanmugam (Vels University, Chennai, India)

P1-2 Formation of silicon nanoclusters in sinx films and their light-emitting properties under the various conditions of deposition and heat treatment
Togambayeva Altynay, F. Komarov, L. Vlasukova, L. Toganbayeva, N. Ankusheva, T. Murat (Al-Farabi Kazakh National University, Republic of Kazakhstan), (Belarusian State University, Republic of Belarus)

P1-3 Nano-encapsulation of short peptides using electrospraying techniques
Rasekh Manoochehr, M. Roldo, E. Barbu, J. Leprince, D. Vaudry, D. Gorecki (School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK)

P2-1 Matpuf: a job-exposure matrix to unintentional nanoscale particles
Sabyne Audignon, A. Lacourt, C. Gramond, S. Ducamp, M. Rinaldo, P. Brochard (Université de Bordeaux, France), (Institut de Veille Sanitaire, France)

P2-2 Proposed structure for information recording of analytical electron microscopy analysis for a nano exposure and contextual information database
Delphine Bard, G. Burdett, M. Mattenklott, J. Pelzer, W. Stöppelmann, C. Schumacher, P. C Tromp, W. Fransman, D. Brouwer, T. Tuomi, T. Kanerva, A. Säämänen, I. Koponen, O. Witschger, I. Koponen, E. Jankowska (HSL, UK), (IFA, Germany), (TNO, Netherlands), (FIOH, Finland) (INRS, France), (NRCWE, Denmark) (CIOP-PIB, Poland)

P2-3 Development of a nano exposure and contextual information database (necid)
Wouter Fransman, J. Pelzer, W. Stöppelmann, D.Brouwer, I. Koponen, D. Bard, O. Witschger, A. Zugasti, E. Jankowska, A. Säämänen, M. Berges (TNO, Netherlands), (IFA, Germany), (NRCWE, Denmark), (HSL, UK), (INRS, France), (INSHT, Spain), (CIOP, Poland), (FIOH, Finland)

P2-4 Detection of carbon nanotubes and carbon nanodiscs on workplace surfaces in a small-scale producer
Maria Hedmer, L. Ludvigsson, C. Isaxon, P. Nilsson, V. Skaug, M. Bohgard, J. H. Pagels, M. E. Messing, and H. Tinnerberg (Occupational and Environmental Medicine, Lund University, Sweden), (Solid State Physics, Lund University, Sweden), (Ergonomics and Aerosol Technology, Lund University, Sweden), (National Institute of Occupational Health, Norway)

P2-5 Development of exposure assessment method with the chamber
Kato Nobuyuki, Y. Koyama, H. Yokoyama, Y. Matsui, M. Yoneda (Kyoto University, JAPAN)

P2-6 Evaluation of Dust in the Working Environment of Toner Handling Plants

Hiroko Kitamura, M. Hasegawa, A. Ogami, T. Myojo (Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan)

P2-7 Exposure assessment of nanoproducts and nanocomposits using chamber method

Matsui Yasuto, N. Kato, Y. Koyama, Y. Shimada, M. Yoneda (Kyoto University, Japan)

P2-8 Exposure assessment of mwcnts in their life cycle

Ono-ogasawara Mariko, M. Takaya, Maromu Yamada (Japan National Institute of Occupational Safety and Health, Japan)

P2-9 Assessing occupational exposure to multi-walled carbon nanotubes: available measurement data, recommended limits and control banding analyses

Anita Radovnikovic, L. Stecca, V. Reina, V. Amenta, K. Aschberger (IHCP-Chemical Assessment and Testing), (IHCP-Molecular Biology and Genomics), (IHCP-Nanobiosciences, Italy)

P2-10 An investigation regarding human responses to toner exposure in a toner manufacturing plant

Hasegawa Masayuki, H. Kitamura, A. Ogami, T. Myojo (Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan)

P2-11 Dispersion state of SiO2 food additiveves in gastrointestinal environment

Retamal Marín Rodrigo Renato, F. Babick, M. Stintz (Technische Universität Dresden, Germany)

P3a-1 Towards an indicator of nanomaterial deposition in the human lung

Dimitrios Bitounis, C. Guibert, V. Forest, D. Boudard, J. Pourchez, J. M. Vergnon, M. Cottier (LINA, France), (Pneumology and Histology-Cytology Departments - Central University Hospital, France)

P3a-2 Development and validation of an inhalation system suitable for rodent exposure to nanoaerosols

Frédéric Cosnier, S. Bau, C. Brochard, S. Grossmann, H. Nunge, R. Payet, S. Michaux, O. Witschger, M. Chalansonnet, L. Gaté (INRS, France)

P3a-3 Measuring at relevant concentrations - Radiolabelling as a versatile tool for sensitive nanoparticle detection

Stefan Schymura, H. Hildebrand, M. Dalmiglio, U. Holzwarth, N. Gibson, K. Franke (HZDR, Institute of Resource Ecology, Germany), (JRC, Institute of Health and Consumer Protection, Italy), (HZDR, Institute of Radiopharmacy, Germany)

P3b-1 Quantitative measurement of carbon nanotubes released from their composites by thermal carbon analysis

Ogura Isamu, M. Shigeta, M. Kotake, M. Uejima, K. Honda (National Institute of Advanced Industrial Science and Technology AIST, Japan), (Technology Research Association for Single Wall Carbon Nanotubes, TASC, Japan)

P3b-2 Evaluation of the behaviour of some sulphonylhydrazone and n-acylhydrazone derivatives as drug delivering systems for the treatment of diabetes mellitus type 2 and cancer

Ferreira Fabio Furlan, A. Laura Ibiapino, L. Pires de Figueiredo, F. Nascimento Costa, E.J. Barreiro, L. Moreira Lima, D. Nascimento do Amaral (Center of Natural and Human Sciences, CCNH, Federal University of ABC UFABC, Brazil)

P3c-1 Club nanoMétrologie: A French initiative to improve the reliability of measurements at the nanoscale

Georges Favre, D. Bernard, F. Piquemal, K. Aguir, S. Cassette, J. Carimalo, Y. De Wilde, S. Ducourtieux, N. Feltin, B. Gautier, P. Lambert, A. Levenson, G. Louarn, T. Macé, P.Maillot, J.M. Moschetta (LNE, France)

P3c-2 A combination of optical and electrochemical transduction principles merged in a novel sensorsystem

Julia Widmaier, D. Furin, F. Kolarov, P. Fechner, G. Proll, B. Sethson, G. Gauglitz (University of Tübingen, DE)

P3c-3 Performance on the vortex shaker dustiness test method as a continuous aerosol generator: time variations in particle number concentration and size distribution of aerosolized nano-tio2

Yamada Maromu, M. Takaya, I. Ogura (Japan National Institute of Occupational Safety and Health, Japan)

P4-1 In vitro evaluation of nickel oxide nanoparticle's toxicity

Mahmoud Abudayyak, T. Gurkaynak Altincekic and G. Özhan (Department of Pharmaceutical Toxicology, Istanbul University, Turkey)

P4-2 Nitric oxide-releasing polymeric nanoparticles against trypanosoma cruzi

Ameda Seabra, N.A. Kitice, C.A.C. Lancheros, S.F. Yamada-Ogatta (Universidade Federal de São Paulo, Exact and Earth Sciences Departament, Rua São Nicolau, Brazil)

P4-3 Chronic exposure of mouse to silica or titanium nanoparticules through drinking water results in renal amyloidosis

Anna Bencsik, D. Boudard, M. Leboidre, B. Laurent, N. Sturm, A. Couderc, L. Lakhdar, P.N. Marche, M. Cottier (Unité Maladies Neurodégénératives, Anses, France)

P4-4 In vitro toxicity of nanoceria: effect of coating and stability in biofluids

Jean-François Berret, Ould-Moussa, M. Safi, M.-A. Guedeau-Boudeville, D. Montero and H. Conjeaud (Matière et Systèmes Complexes, UMR 7057 CNRS Université Denis Diderot, France)

P4-5 Mechanisms of TiO2 nanoparticles genotoxicity: impact on DNA repair in, A549 and BEAS-2B Epithelial Pulmonary Cells

Mathilde Biola-Clier, M. Line Jugan, L. Armand, J.C. Gaillard, J. Armengaud, S. Sauvaigo, N. Herlin-Boime, T. Douki, M. Carriere (Université Grenoble-Alpes, INAC, SCIB, LAN, France), (CEA, INAC, SCIB, LAN, France)

P4-6 Deeper penetration of TiO2 nanoparticles in neoplastic vs. normal human oral mucosa models

Eivind Birkeland, V. Konstantinova, M. Ibrahim, M.C. Marthinussen, D.E. Costea, M.R. Cimpan (Faculty of Medicine and Dentistry, University of Bergen, Norway)

P4-7 Metal homeostasis perturbations induced by ZnO nanoparticles in hepatocyte cells

Mireille Chevallet, K. Um, P. Charbonnier, P. Henri Jouneau, E. Mintz and I. Michaud-Soret (LCBM UMR5249 UJF CNRS CEA, France)

P4-8 Role of autophagy in response to titanium dioxide nanoparticles

Vanessa Cohignac, A. Gerdil, N. Herlin, J. Boczkowski, J.C. Pairon, S. Lanone (Inserm, France)

P4-9 Silver nanoparticles cytotoxicity – viability and apoptosis effects to a keratinocyte cell line

Verónica Isabel Correia Bastos, J.Miguel P Ferreira de Oliveira, L. F. Duarte, C. Santos and H. Oliveira (CESAM & Laboratory of Biotechnology and Cytomics, Department of Biology, University of Aveiro, Portugal)

P4-10 The effectof different sizes and doses of nano particle zinc on some oxidative stress parameters in rats

Mina Danesh, M. Hejazi, M. Rezayat, M. Kazem Koohi (IAUPS _ Tehran)

P4-11 E171 food additive and titanium dioxide nanoparticule toxicity on intestine cell models

Marie Dorier, E. Brun, F. Barreau, N. Herlin-Boime, M. Carrière (Université Grenoble Alpes, INAC, SCIB, France, CEA)

P4-12 Is p25 a realistic model to study the toxicity of tio2 in the gastro-intestinal tract?

William Dudefoi, H. Terrisse, B. Humbert, M. Hélène Ropers (INRA, Biopolymères Interactions Assemblages, France)

P4-13 Graphene oxide sheets-based platform for induced pluripotent stem cells culture: toxicity, adherence, growth and application

Nelson Duran, M. Durán, P.F. Andrade, A.C.M. Luzo, W.J. Fávaro (NanoBioss, UNICAMP, Brazil), (Biol. Chem. Lab. UNICAMP, Brazil)

P4-14 Shyntesis, characterization and citotoxicity evaluation of nitric oxide-iron oxide magnetic nanoparticles

Paula Silvia Haddad, T.N. Britos, M.C. Santos, A.B. Seabra, M.V. Palladino, G.Z. Justo (Exact and Earth Sciences Department, Universidade Federal de São Paulo, Brazil)

P4-15 Analytical characterization of silver nanoparticles and proteomic responses in human caco-2 cells after oral ingestion

Hansen Ulf, A. Thuenemann, A. Lampen (Federal Institute for Materials Research and Testing BAM + Unter den Eichen 87, Germany)

P4-16 Hsp70 as an indicator of stress in the cells after contact with nanoparticles

Šárka Hradilová, M. Havrdová, A. Panáček, L. Kvítek, R. Zbořil (Regional Centre of Advanced Technologies and Materials, Faculty of Science, Czech Republic)

P4-17 Prediction of nano-particle permeation through pulmonary alveolar epithelia based on integrated uses of a cell-based in vitro model and a numerical simulation

Kokoro Iwasawa, K. Harano, R. Ogasawara, T. Aoyama, N. Shinohara, G. Zhang, M. Gamo, A. Suwabe, Y. Sakai (Institute of Industrial Science, the University of Tokyo, Japan)

P4-18 Investigation of the potential cytotoxic effects of zinc oxide nanoparticles

Ayşegül Karapınar Mantu, B. Pütün, M. Abudayyak (Özel Cevizlibağ Doğa Anadolu Lisesi, Turkey)

P4-19 Characterization of Copaxone® by Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS)

Tatiana Molotsky, R. Krispin, T. Hasson and A. Komlosh (Analytical Development, Discovery & Product development, Global R&D, Teva Pharmaceutical Industries Ltd, Israel)

P4-20 Exposure to manufactured nanoparticles during gestation: impact on the respiratory tract of the offspring in a mouse model

Paul Emmanuel, J. Rose, J. Boczkowski, S. Lanone, C. Delacourt, J.C. Pairon (Inserm U955, faculté de Médecine, France)

P4-21 Chitosan nanoparticles; assessment of internalization and cytotoxicity in vitro
Piña Olmos S, Díaz Torres R., Ramírez Noguera P (Labartorio de Toxicologia celular-Unidad de Investigación Multidisciplinaria, Facultad de Estudios Superiores Cuautitlán, México)

P4-22 What effects have fine particles in the vascular system? an integrated proteomic and metabolomic study on human endothelial cell
Mario Pink, N. Verma, A. Rettenmeier, S. Schmitz-Spanke (Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Germany)

P4-23 Cyto and Genotoxicity of AgNP on MG-63 and A549 cell lines
Rosário Fernanda, C. Reis, C. Santos, H. Ovileira (CESAM – Centre for Environmental and Marine studies, University of Aveiro, Portugal), (Laboratory of Biotechnology and Cytomics - Department of Biology, University of Aveiro, Portugal)

P4-24 Toxicological effects of TiO2 nanoparticles: influence of nanoparticles characteristics and cellular models
Gladys Saez, Q. Le Trequesser, G. Devès, P. Barberet, C. Michelet, M.H. Delville, H. Seznec (Université de Bordeaux, Centre Etudes Nucléaires de Bordeaux Gradignan, France), (CNRS, IN2P3, Centre Etudes Nucléaires de Bordeaux Gradignan, France)

P4-25 In vitro evaluation of iron oxyde nanoparticles and titanate nanotubes on a hepatoma cell line : cytotoxicity and genotoxicity
Yasmine Saibi, V. Bellat, I. Séverin, J. Boudon, N. Millot, M.C. Chagnon (Welience, Maison Régionale de L'Innovation / Laboratoire Interdisciplinaire Carnot de Bourgogne, France)

P4-26 In vivo nanotoxicology of hybrid systems based on copolymer/silica nanoparticles/anticancer drug
Camila P. Silveira, A. J. Paula, L. M. Apolinário, W. J. Fávaro, N. Durán (Chemistry Institute, Universidade Estadual de Campinas UNICAMP, Brazil)

P4-27 Screening platform for human health impact from inhalation of airborne nanoparticles
Sandra Verstraelen, E. Frijns, I. Nelissen (Flemish Institute for Technological Research, Environmental Risk and Health Unit, Belgium)

P4-28 Construction of a database on nanotoxicity from peer reviewed publications: data curation and implementation of ontology
Hanne Vriens, D. Mertens, T. Wittenberger, P. Hoet (KU Leuven, Faculty of Medicine, Department of Public Health and Primary Care, Belgium)

P4-29 Binding and uptake mechanisms of charged gold nanoparticles in immune cells
Mirjam Zimmermann, M. Boyles, A. Duschl (University of Salzburg, Department of Molecular Biology)

P4-30 The "New" old Dose concept for nanoparticles risk assessment
Myrtill Simkó, D. Nosske, Wolfgang G. Kreyling (Austrian Institute of Technology GmbH, Health and Environment Department,, Austria)

P4-31 Toxicity of pesticides and nanomaterials to neutrophils cells
Yubing Pu, B. Laratte, R.S. Marks, R. E. Ionescu (Laboratoire de Nanotechnologie et d'Instrumentation Optique, Institut Charles Delaunay, Université de Technologie de Troyes, France)

P5-1 Interactions and toxicology of silver nanoparticles in aquatic ecosystems
Ester Artells, C. Levard, J. Issartel, M. Auffan, A. Thiéry (IMBE UMR-CNRS Université d'Avignon, France)

P5-2 Size-dependent toxicity of barium titanate to chlorella vulgaris

Roberta Brayner, H. C. Polonini, H. M. Brandão, N. R. B. Raposo, M. Antônio, F. Brandão, L. Mouton, A. Couté, C. Yéprémian, Y. Sivry (Interfaces, Traitements, Organisation et Dynamique des Systèmes ITODYS, Université Paris Diderot, France)

P5-3 Silver nanoparticle toxicity to pseudomonas putida monospecies biofilms under flow conditions

Florian Mallevre, T. F. Fernandes, T. J. Aspray (School of Life Sciences, NanoSafety Research Group, Heriot-Watt University, UK)

P5-4 Carbon nanotubes enhanced the lead toxicity on the freshwater fish: histopathological effects in the gills

Diego Stéfani T. Martinez, J. Campos-Garcia, K. F. O. Rezende, J. R. M. C. Silva, O. L. Alves and E. Barbieri (LNNano - Brazilian Nanotechnology National Laboratory, CNPEM - Center on Research in Energy and Materials, Brazil)

P5-5 Fate and behavior of silver nanoparticles in simple and complex matrices

André Nogowski, R. Renato Retamal Marín, M. Stintz (TU Dresden, Institute of Process Engineering and Environmental Technology, Research Group Mechanical Process Engineering, Germany)

P5-6 Lichens as biomonitors of cnt aerossols: a possibility?

Camila de Oliveira Viana, A. Pinheiro Santos, L.O. Ladeira, A. Correa Junior (Departamento de Microbiologia, Universidade Federal de Minas Gerais, Brasil)

P5-7 Fate and transport of engineered nanoparticles along the exposure pathway wastewater – sludge – plant

Heike Hildebrand, S. Schymura, P. Schneider, T. Lange, T. Fricke, K. Ziegler, K. Franke (Helmholtz-Zentrum Dresden-Rossendorf, Germany)

P5-8 Nanomaterials as potentially safer alternative to flame retardants of concern – a comparative hazard assessment

Karin Aschberger, V. Amenta, A. Christou, J. Muller, Laia Q. Pesudo, A. Radovnikovic, L. Stecca, A. A Stec (IHCP-Nanobiosciences, Italy)

P5-9 Chronic contamination of aquatic mesocosms by ceo2 nanoparticles with different surface properties

Marie Tella, M. Auffan, A. Thiéry, C. Santaella, L. Brousset, E. Morel, C. Pailles, J. Issartel, W. Achouak, B. Angeletti P. Chaurand, J. Rose, Mark R. Wiesner, J-Y Bottero (CNRS, Aix-Marseille Université, CEREGE UM34, UMR 7330, Aix en Provence, France)

P5-10 Ecotoxicology study of main nanofillers used in packaging materials

Eva Araque, C. Fito, O. Andreu-Sánchez (Packaging, Transport & Logistics Research Institute, Spain)

P5-11 Interaction of carbon nanotube and cellulose nanofiber with algal cells klebsormidium flaccidum

M. M. Pereira, L. Mouton, C. Yéprémian, A. Couté, J. Lo, J. M. Marconcini, L.O. Ladeira, N. RB Raposo, H. M. Brandão and Roberta Brayner (UFJF, Brazil)

P6-1 Evaluation of the influence of nano-objects in the reaction to fire properties of construction products exposed to accidental fire

Aitor Barrio Ulanga, C. Vaquero Moralejo, JL. De Ipiña, *(TECNALIA R&I, C/Geldo, Spain)*

P6-2 Technologies to simulate the release of engineered nanomaterials (enms) from polymeric nanocomposites due to mechanical processes

Ainhoa Egizabal, M. Blázquez, I. Unzueta, C. Elizetxea (TECNALIA Research and Innovation, Spain)

P6-3 Nanoparticle release quantification during low and high energetic dry dispersing of nanostructured powders

Daniel Göhler, M. Stintz (Research Group Mechanical Process Engineering, Institute of Process Engineering, Technische Universität Dresden, Germany)

P6-4 Characterization of nanoparticulate emissions from the incineration of wastes containing manufactured nanomaterials

Olivier Le Bihan, D.T. Tran, G. Ounoughene, D. Venditti, S. Durecu, A. Joubert, E. Fiani, T. Meunier, B. Debray, L. Le Coq (INERIS, France)

P6-5 Behavior and fate of halloysite nanotubes (hnts) when incinerating pa6/hnts nanocomposite

G. Ounoughene, O. Le Bihan, C. Chivas-Joly, C. Motzkus, C. Longuet, B. Debray, A. Joubert, J-M. Lopez-Cuesta, L. Le Coq (LUNAM, Ecole des Mines de Nantes, GEPEA, CNRS, France), (C2MA, Ecole des Mines d'Alès, France), (ADEME, France)

P6-6 Study of nanoparticles due to the emission of polyurethane foam in real condition of use

Eric Zimmermann, H. Fontaine, D. Locatelli, S. Cetre, P. Charléty (Univ. Grenoble Alpes, PNS, CEA, France)

P6-7 Dustiness of bulk nanomaterial powders using the vortex shaker method

Olivier Witschger, S. Bau, R. Payet, B. Bianchi, K. Nzambangoye
INRS, France)

P7-1 Recommendations for a nanosafe production of nano-device involved in inflammatory disorders treatment

Christophe Bressot, N. Shandilya, O. Le Bihan, O. Aguerre-Chariol (INERIS, France)

P7-2 Safety by molecular Design: NANO CuO as case study

L. Viale, A.L. Costa (ISTEC – CNR, Italy)

P8 Human toxicity and freshwater ecotoxicity characterisation factors for engineered nanoparticles : toward a spatial differentation

Beatrice Salieri, R. Hischier, S. Righi, A. Pasteris, S. Irving Olsen, (Empa, Swiss Federal Laboratories for Materials Science and Technology, Switzerland)

P9-1 Development of a technical specification: guidelines for the management and disposal of waste from the manufacturing and processing of manufactured nano-objects

Delphine Bard, D. Koltsov, R. Hawkins (HSL, UK)

P9-2 Grouping of nanomaterial by health, safety & environmental characteristics

Christian Schumacher, J. Pelzer (Institute of Occupational Safety and Health of the German Social Accident Insurance IFA, Germany)

P9-3 CEN/TC 352/WG3/PG3 protocols for determining the explosivity and flammability of powders containing nano-objects (for transport, handling and storage)

Julien Porcher, A. Vignes, B. Debray, A. Janès, D. Carson, E. Frejafon, J. Bouillard (INERIS, France)

P9-4 Reference nanoparticles or reference protocol: what should come first to make significant progress in health risk assessment?

Claude Emond, G. Johanson, O. Witschger, P. Hoet, and G. Oberdorster (BioSimulation Consulting Inc, USA)

P11-1 Human risk assessment and its application to nanotechnology: a challenge for the assessor

Claude Emond, L. Multigner (University of Montreal, Department of Environmental and Occupational Health, Canada), (BioSimulation Consulting Inc, United States)

P11-2 Life nanorisk – best practices, effectiveness, prevention and protection measures for risk control posed by engineered nanomaterials

Evelien Frijns, P. Berghmans, C. Fito, E. de la Cruz, G. Boulougouris, M. Santamaria, S. Padovani, F. Marcori, S. Priante, P. Beltran, E. Santamaria, M. Perez, J. Perez, J. Gomez, P. Caceres (VITO NV, Flemish Institute for Technological Research, Belgium)

P11-3 REACHnano Tool: a new web based toolkit to support the chemical safety assessment of nanomaterials

George Boulougouris, C. Fito, J. de Dios Diaz (Instituto Tecnológico del Embalaje, Transporte y Logística, Spain)

P12-1 Regulation and innovation dynamics for nanoresponsible development: the case of the french code de l'environnement, l 523-1 to l 523-5

Claire Auplat, S. Ben Slimane (Novancia Business School Paris, France)

P12-2 The DaNa2.0 Knowledge Base Nanomaterials – Communicating Current Nanosafety Research

Clarissa Marquardt, Harald F. Krug, D. Kuehnel, F. Paul, C. Steinbach, K. Nau (Karlsruhe Institute of Technology, Germany)

P12-3 Nanotechnology regulation: multilateral initiatives for a responsible and beneficial development of nanoproducts

Pedro Canisio Binsfeld (Brazilian Health Surveillance Agency, ANVISA, Brazil)

P12-4 Class action litigation for skin cancer by sunscreens

Thomas Prevenslik (QED Radiations, China)

P12-5 Omnt, a strategic watch organization

Emma Richet, S. Berger (OMNT, Campus Minatec CEA, France)

CONFERENCE OPENING – PL0d

CHALLENGES AND PROMISING STRATEGIES FOR FABRICATING AND USING NANOMATERIAL-ENABLED MEMBRANES FOR WATER TREATMENT

Mark Wiesner, Duke University

Membrane technologies represent a wide range of solutions for separations required in municipal water treatment. Their small footprint, ability to automate and ability to remove materials form salts to microbes, makes them attractive for both conventional water treatment as well as reuse. This talk surveys some recent developments in membrane technologies as enabled by nanomaterials for the purposes of desalination, membrane disinfection and water reuse. Challenges associated with some strategies for using nanomaterials to enhance membranes processes will be reviewed and promising applications will be highlighted.

CONFERENCE OPENING – PL0e

FROM NANOMEDICINE TO NANOSAFETY: A JOURNEY INTO NANOCHARACTERISATION

Patrick Boisseau, Business Development Nanomedicine, Microtechnologies for Biology and Healthcare Division, Léti, France

Mankind is still fighting against a high number of serious and complex illnesses like cancer, cardiovascular diseases, multiple sclerosis, Alzheimer's and Parkinson's disease, and diabetes as well as different kinds of serious inflammatory or infectious diseases (e.g. HIV). Most of theses diseases have a tremendous negative impact not only on the patient himself but also on the whole society and linked social and insurance systems. It is of utmost importance to face these plagues with appropriate means.

Nanomedicine, the application of nanotechnology to health, raises high expectations for millions of patients for better, more efficient and affordable healthcare and has the potential of delivering promising solutions to many illnesses. Research in nanomedicine will allow for a better understanding of the functioning of the human body at molecular and nanometric level and it will thus give us the possibility to intervene better at pre-symptomatic, acute or chronic stage of illnesses.

Several areas of medical care are already benefiting from the advantages that nanotechnology can offer. The first nanotechnology-based targeted drug delivery systems are already on the market, others are in clinical trials or, by far the largest part, are under development. Another highly attractive area of nanomedicine is diagnostics at nanoscale. The aim is to identify a disease at the earliest possible stage. Ideally already a single cell with ill behaviour would be detected and cured or eliminated. New concepts for regenerative medicine give hope to many patients with organ failure or severe injuries. Already today artificial skin, bone and cartilage are in an advanced stage of development and partly already on the market.

The promising possibilities that nanomedicine might offer in the future have to be counterweighted against possible risks of this new technology. It is of utmost importance to examine upfront with care and responsibility its possible side effects to human beings and the environment. Several European projects are already dealing with this highly important issue. Also ethical concerns have to be taken into account. It may also be necessary to examine existing legislation for its applicability to nanomedicine.

Industry has increasing interest in stepping into the area of nanomedicine and the expected market share of final products is expected to be significant. In addition to the improved quality of health care, the creation of new jobs can be expected.

N° PL1

NANOPARTICLES PROPERTIES AND INTEREST FOR INDUSTRIAL APPLICATIONS

Francois Tardif, O. Poncelet, P. Tiquet (Université Grenoble Alpes, Commissariat à l'Energie Atomique et aux Energies Alternatives, Plateform Nano Safety, France)

The challenges and responsibilities of the Nanosafety community are at a level that meets the nanomaterial perspectives in terms of benefits for human civilization. Indeed, a dynamic development of nanomaterials requires the adhesion from the public at large who rightly demand progress in Nanosafety as a prerequisite.

Making mineral resources more efficient for a better sustainable sharing all across the globe, increasing the yields of new energy technologies, enabling drugs that act selectively and locally are just few examples through the large actual positive applications of nanomaterials in progress today for humanity.

In addition of today's enthusiastic perspectives, nanomaterials represent also an extraordinary field of brand new innovation for the future for which our imagination is often our only limit. Indeed, sophisticated bottom up synthesis processes are already available, enabling through a kind of molecular Lego® construction game, to achieve very specific or complex shapes able to lead to actually Smart active materials.

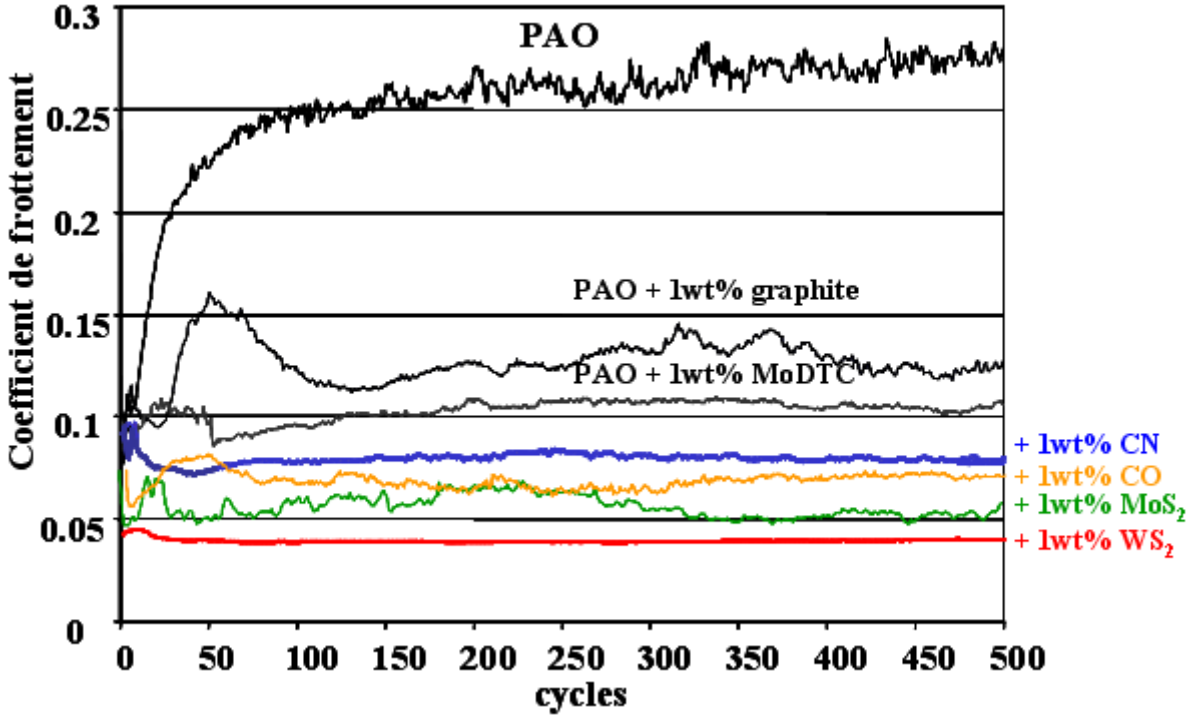
This talk deals with a physical description of the different families of advanced nanomaterials followed by the ways to use them in order to give new functionalities to tomorrow's materials.

N° O1a-1

NANOPARTICLES: POTENTIAL ADDITIVES FOR SUSTAINABLE LUBRICATION

Fabrice Dassenoy[1], (1) Ecole Centrale de Lyon – Laboratoire de Tribologie et Dynamique des Systèmes, 36 Avenue Guy de Collongue, 69134 Ecully, cedex, France

The technology involved in lubrication by nanoparticle is a rapidly developing scientific area and one that has been watched with interest for the past ten years. These tiny particles present several major advantages compared to organic molecules currently used as lubricant additives: their chemical inertness, their nanometer size which allows them to enter easily the contact area, their efficiency at ambient temperature …).
In this presentation, we will focus on the tribological properties of Inorganic Fullerene (IF) like nanoparticles made of metal dichalcogenides when used as lubricant additives. Parameters (particle properties, test conditions …) having an influence on the lubricating properties of the nanoparticles will be discussed. Their lubrication mechanism(s) will be also presented in detail as well as the recent developments in i) the characterisation of the tribological properties of the nanoparticles and ii) their manipulation at a nanoscale. Finally, we will discuss the efforts to be made in the future to overcome the technological barriers involved in the development of a new generation of lubricants incorporating nanoparticles.



Friction curves obtained from different nanoparticles based dispersions (CN: carbon nanotube, CO: carbon onion, IF-MoS_2 and IF-WS_2. Comparison with the pure base oil (PAO) and the behavior of traditional additives (graphite and MoDTC).Tribological test performed with a pin on flat tribometer (steel substrate, contact pressure of 0.83GPa, ambient T°C).

N° O1a-2

PROSPECTS AND 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 utrophication, 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 devolopment 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 physi-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, bioaccumulationa 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 absorption, transport, accumulation, degradation, etc, especially the transportation and accumulation towards the edible organs and metabolism increasing toxic phenomenon.

N° O1a-3

NANOMATERIALS AS A NEW APPROACH TO FIRE-RETARDANCY

Fiona Hewitt, Diana Suleiman Eid Rbehat[2], Artur Witkowski, Anna Stec and T.R. Hull, University of Central Lancashire, Preston, Lancashire, PR1 2HE, U.K.

The replacement of natural materials with highly flammable synthetic polymers has led to the requirement for fire retardants to reduce the deaths and damage caused by fires. The most widely used fire retardants have been based on chlorinated or brominated compounds, however, their acute toxicity and long-term health and environmental effects have raised concern so there is now work being undertaken to replace these with safer alternatives. Much interest has been shown in incorporating various nanomaterials into polymer matrices in order to benefit from the impressive physical properties. Receiving particular attention are carbon nanotubes (CNTs), nanoclays and graphene. These materials work in different ways to reduce the flammability, heat release and time to ignition of burning polymeric materials. The greatest effect on fire behaviour is observed when nanomaterials are used in combination with other fire-retardant additives, such as mineral fillers like aluminium hydroxide (ATH). Mineral fillers are widely used non-halogenated fire-retardants as they are cost effective and safer environmentally. Their main problem is their detrimental effect on the desirable properties of polymers (*e.g.* strength and flexibility) resulting from the high loadings required to achieve satisfactory fire behaviour. However, the addition of nanomaterials, even at low loadings (<5%) can help to redeem the mechanical properties of these formulations, while reducing the necessary loading of mineral filler. The presence of nanomaterials allows the overall filler loading to be reduced, while maintaining fire retardant properties. This is clearly beneficial in producing polymeric materials that have acceptable fire-behaviour and remain fit for purpose. However, such complex, multicomponent systems, containing, for example, a polymer, two fire retardants and a nanoscopic material require painstaking formulation to optimise their physical and fire properties.

Pyrolysis modelling is a useful tool for predicting the behaviour of materials in fires. Models have been developed to describe the thermal decomposition of virgin polymers, however, very little is understood on the most accurate ways of implementing fire-retardant effects into models. Pyrolysis modelling is developing rapidly and is being applied as part of a screening procedure when developing new formulations, saving time, money and sample volume.

This poster details the physical changes that take place in the matrices of polymer-nanocomposites as they thermally decompose. The effects of these changes on flammability, heat release and time to ignition are discussed, and possibilities for implementing these changes into pyrolysis models are considered.

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n0 308391.

N° O1a-4

SUPER-STRONG NANO-COMPOSITE MATERIALS FOR BUNKER & COMMAND POST IN ARMY

Dr Dalvinder Singh Grewal
Dean R & D
Desh Bhagat University

Nano-composites from steel and polymers can be developed as stronger materials to withstand the impact of bullets and bombs. Nano composites dispersed with carbon nano-tubes in polymers or steel enhance mechanical strength and higher temperature resistance. Stronger polymers will also provide observation from inside for the purpose of observation and fire. Polymers or steel are melted in a crucible/mould of desired shape of bunker and command posts walls and dispersed with carbon nano-tubes at the melting temperature of polymer/steel and frozen up to room temperature to yield the nano-composites.

N° O1a-5

THE IN VIVO ACTIVATION OF PERSISTENT NANOPHOSPHORS FOR OPTICAL IMAGING OF VASCULARIZATION, TUMOURS AND GRAFTED CELLS

Maldiney T, Bessière A, Seguin J, Teston E, Sharma SK, Viana B, Bos AJ, Dorenbos P, Bessodes M, Gourier D, Scherman D, Richard C.

Optical imaging for biological applications requires more sensitive tools. Near-infrared persistent luminescence nanoparticles enable highly sensitive in vivo optical detection and complete avoidance of tissue autofluorescence. However, the actual generation of persistent luminescence nanoparticles necessitates ex vivo activation before systemic administration, which prevents long-term imaging in living animals. Here, we introduce a new generation of optical nanoprobes, based on chromium-doped zinc gallate, whose persistent luminescence can be activated in vivo through living tissues using highly penetrating low-energy red photons. Surface functionalization of this photonic probe can be adjusted to favour multiple biomedical applications such as tumour targeting. Notably, we show that cells can endocytose these nanoparticles in vitro and that, after intravenous injection, we can track labelled cells in vivo and follow their biodistribution by a simple whole animal optical detection, opening new perspectives for cell therapy research and for a variety of diagnosis applications.

N° O1a-6

EXPLORATION OF ACTIVATION ENERGY AND ELECTRICAL APPLICATIONS OF SYNTHESIZED AL DOPED ZNO NANOMATERIALS AS HUMIDITY/GAS NANOSENSORS

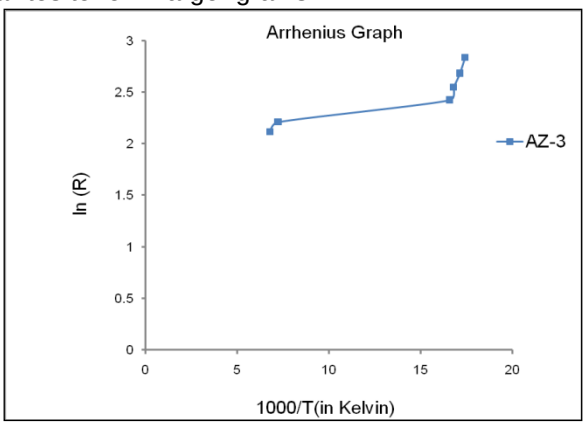
Suneet Kumar Misra[1], N.K. Pandey[1] and Vandna Shakya[1], (1) Sensors and Materials Research Laboratory, Department of Physics, University of Lucknow, Lucknow, India-226007 *E-mail: suneetm31@gmail.com*

Analysis on the electrical applications of the nanomaterials is very important as these nanomaterials are used in the development of the gas/humidity sensors. Activation energy gives us a deep understanding of the electrical properties of the nanomaterials. Zinc Oxide (ZnO) is one of the most important metal oxides having various applications in gas/humidity sensors. ZnO is a very versatile II-VI compound semiconductor with a wide bandgap of about 3.3 eV. Doping is an effective method for manipulating different applications of semiconductors. A suitable doping element is chosen to enhance the electrical and optical properties of ZnO. Ceramic humidity/gas sensors have attracted much attention due to their chemical and physical stability.

In the present work, sol-gel method is used for obtaining synthesized Al doped ZnO nanocrystalline powders. In the experiments, zinc acetate (Zn $(CH_3COO)_2.2H_2O$) is used as starting material, methanol as organic solvent and mono ethanol ammine as surfactant. Aluminium sulphate is used for doping Al. All the sols were mixed in appropriate proportions so that there is 3% Al doping in ZnO. Prepared nanopowder was given pellet shape by applying pressure of 300 MPa. Pellets were annealed in air at different temperatures from 300ºC to 600ºC for 3 hours. Pellet samples made were labeled AZ-3 (3% Al doped in ZnO).

The activation energy values for sample AZ-3 from the Arrhenius plot is found to be 0.042 eV for temperature range 80°C to 140°C and 0.0186 eV for temperature range 180°C to 200°C. This sensing element shows lower values of activation energies in the two regions indicating that this sensing element has lower operating temperatures and may be used at room temperature as well. From the V-I characteristics graph of this sensing element, a sudden increase in the current has been observed.

The humidity sensing studies of these samples were also analyzed. The sample AZ-3 annealed at 600ºC is showing the best results with sensitivity of 21.40 MΩ/%RH. This sensing element manifests lower hysteresis, less effect of ageing and high reproducibility for annealing temperature 600°C. The sensitivity increases with the increasing annealing temperature. The response time, recovery time, hysteresis, sensitivity, ageing effects and various other parameters were also studied. The crystallite size from XRD for the sensing element AZ-3 is in the 16-37 nm range. The average grain size as measured from SEM micrograph of this sensing element is found to be 96 nm suggesting agglomeration of the crystallites to form larger grains.



Arrhenius Graph for the sensing element AZ-3

N° O1a-7

APPLICATION OF CARBON NANO-TUBES (CNTs) / ALKYD RESIN COMPOSITES AS ANTICORROSIVE COATING

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Effect of carbon nano-tubes (CNTs) on the corrosion protection of carbon steel coated by alkyd resin and tested after immersion in 3.5 % NaCl solution for different periods was evaluated by electrochemical impedance spectroscopy (EIS) measurements and scanning electron microscopy (SEM) investigations. Changes in the impedance characteristics of the systems were found to be greatly affected by the percentage of CNTs. Degradation of alkyd resin film without CNTs was observed after 72 h. On other hand no blisters, pin-holes and delamination were observed for alkyd resin containing 0.5% CNTs. It was found that CNTs improved the corrosion resistance of alkyd resin.

N° O1a-8

IN VIVO STUDY OF NOVEL NANOCOMPOSITE FOR PROSTATE CANCER TREATMENT

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It is known that anticancer drugs usually trigger a broad range of side effects that affect patients' quality of life. Doxorubicin (DOX), for instance, is an antitumor drug used in treatment of various types of cancer that has its efficacy limited because of its intrinsic toxicity. Of the many alternatives to minimize this issue, we underline the association with nanomaterials as well as with other molecules with different mechanisms of action. The use of nanoparticles as carriers can lead to sustained release and effective drug delivery, thus diminishing toxicity. In turn, Anti-PDE-5 is a molecule that acts inhibiting the enzyme phosphodiesterase-5, which expression is increased in many carcinomas, indicating its potential in tumor progression. It has been demonstrated that DOX association with Anti-PDE-5 leads to a protection effect against cardiac ischemia caused by DOX and it might be due to the apoptosis caused by increased expression of nitric acid synthetase.
To evaluate Anti-PDE-5 potential use in cancer treatment in combination with silica nanoparticles (SiNP), we developed three systems containing Anti-PDE-5 and a biocompatible copolymer - that allows *in situ* gelling - differentiated only by SiNP concentration (Table 1).

Table 1. *[SiNP] in the systems*

System	SiNP (mg/mL)
S1	-
S2	1
S3	5

The systems were tested in rats with chemically induced prostate cancer. Fig.1. shows gel dissolution rates for the three systems, providing controlled release of SiNP and Anti-PDE-5 as the gel dissolves. Histopathological analyses from prostate cells from cancer control group showed a high frequency of tumors level high, while groups treated with a) S1 showed higher frequency of tumors level intermediate with abundance of atypical cells; b) S2 showed higher frequency of tumors level low, with abundance of microacines, indicating inflammation and atrophy and c) S3 showed a decrease in frequency of low-level tumors compared to S2.

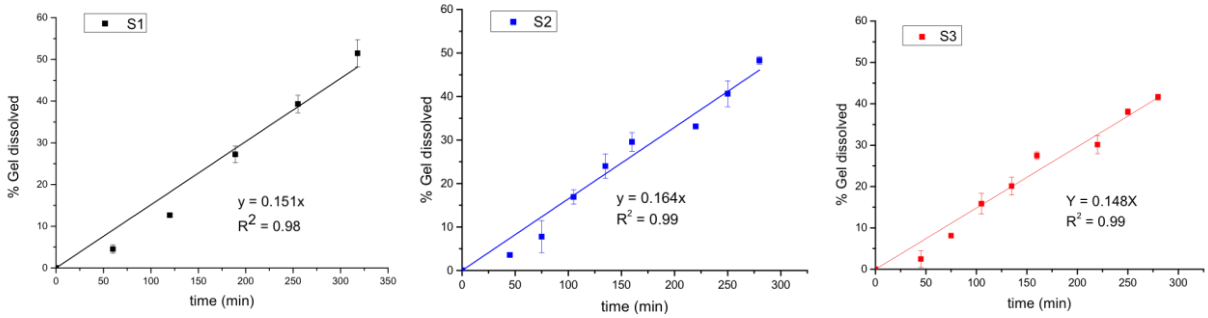

Figure 1. *Gel dissolution*

Histopathological analyses suggest that SiNP play an important role in tumor suppression as tumor level diminished with SiNP introduction as well as with increasing SiNP concentration. The results encourage future association of Anti-PDE-5 with DOX encapsulated in the same particle.

N° O1a-9

PREPARATION, CHARACTERIZATION AND TESTS OF INCORPORATION IN STEM CELLS OF SUPERPARAMAGNETIC IRON OXIDE

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Superparamagnetic iron oxide nanoparticles (SPIONs) have been produced and used as contrast-enhancing agents in magnetic resonance imaging (MRI) for diagnostic use in a wide range of maladies including cardiovascular, neurological disorders, and cancer. The reasons why these SPIONs are attractive for medical purposes are based on their important and unique features. The large surface area of the nanoparticles and their manipulation through an external magnetic field are features that allow their use for carrying a large number of molecules such as biomolecules or drugs. In this scenario, the present work reports on the synthesis and characterization of SPIONs and *in vitro* MRI experiments to increase their capacity as probes for MRI applications on stem cells therapy. Initially, the SPIONs were prepared through the co-precipitation method using ferrous and ferric chlorides in acidic solution. The SPIONs were coated with two thiol molecules such as mercaptosuccinic acid (MSA) and cysteine (cyst) (molar ratio SPIONs:ligand = 1:20), leading to the formation of a stable aqueous dispersion of thiolated nanoparticles (SH-SPIONs).The SH-SPIONs were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and vibrating sample magnetometer (VSM). The results showed that the SH-SPIONs have a mean diameter of 14 nm and display superparamagnetic behavior at room temperature. Preliminary tests of incorporation of SH-SPIONs were evaluated in HeLa cells and stem cells. The results showed that the thiolated nanoparticles have no toxic effects for both cell types and successfully internalized and enhance the contrast in MRI. Figure 1 shows the incorporation of MSA-SPIONs in stem cells for 6 hours.

Figure 1. (a) Stem cells without MSA-SPIONS; (b) Incorporation of MSA-SPIONs in stem cells for 6 hours.
Acknowledgements: FAPESP, CNPq, CAPES.

N° PL2

RECENT DEVELOPMENTS IN HUMAN EXPOSURE ASSESSMENT

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Over the last couple of years the number of published studies on occupational exposure to nanomaterials have increased substantially, documenting relevant developments with respect to release and exposure assessment, exposure modelling, and harmonization and standardization issues. Partly due to lack of harmonization with respect to measurement strategy and data interpretation and reporting, meta-analysis of exposure data is not feasible yet, however, some indications of scenarios with potential for relatively high release and eventually exposure can be observed which enables preliminary mapping of exposure 'hot spots'. In addition to inhalation exposure during direct handling of nanomaterials, exposure due to resuspension of deposited nanoparticles and its agglomerates have been addressed, as well as the relevance of other exposure routes, e.g. dermal and ingestion exposure.
In view of quantitative risk assessment, actually quantification in terms of biologically relevant dose is still in its infant stage, partly due to the ongoing debate on which metric would represent biologically relevance appropriately and partly due to limitations with respect to personal (size –selective) measurement devices.
The paper will present the state-of-the art and will address recent developments in the topics mentioned above.

N° O2a-1

MASS VS NUMBER-BASED EXPOSURE ASSESSMENT TO NANOPARTICLES, A COMPARISON OF A PERSONAL SAMPLER AND MONITORS

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Exposure to engineered nanoparticles through inhalation in workplaces requires careful assessment in view of worker protection, because of the intrinsic ability of nanosized particles to reach and deposit in the deep alveolar region of the lungs. In occupational hygiene, the assessment of exposure to particles traditionally relies on sampling of particles below 10 µm or 4 µm, followed by mass concentration determination. Nanosized particles contribute only negligibly to these mass concentrations, and hence the conventional exposure assessment tools are not sufficiently sensitive to assess the personal exposure to airborne nanoparticles.
Considering this, a novel sampling and analysis tool was developed to provide an accurate mass-based assessment of personal exposure to nanoparticles. The developed personal sampler collects airborne nanoparticles located in the breathing zone of the worker onto filters, which are then analyzed by X-ray fluorescence spectroscopy. This highly-sensitive technique yields the elemental composition of the collected particles and their average mass concentration in air. This strategy allows a detection limit in the tens of ng/m^3 over a complete work shift (8h) for the elements of interest. The mass concentration has been compared with the particle number concentration given by conventional particle counters by means of measurements of the average effective density of the sampled particles. The performance of the sampler was evaluated on mono- and polydisperse aerosols generated in a controlled environment. The comparability between mass-based and number-based exposure assessments will be discussed.
This work was performed within the framework of the nanoIndEx FP7 project. nanoIndEx is supported by the French National Funding Agency for Research (ANR), the German Federal Ministry of Education and Research (BMBF), the British Technology Strategy Board (TSB) and the Swiss TEMAS AG, under the frame of SIINN, the ERA-NET for a Safe Implementation of Innovative Nanoscience and Nanotechnology.

N° O2a-2

ANALYSIS AND CHARACTERIZATION OF MULTIVARIATE STOCHASTIC SIGNALS SAMPLED BY ON-LINE PARTICLE ANALYSERS. APPLICATION TO THE QUANTITATIVE ASSESSMENT OF EXPOSURE TO NOAA IN OCCUPATIONAL SCENARIOS.

Jesús López de Ipiña[1], Celina Vaquero[1], Cristina Gutierrez-Cañas[2], David Y. H. Pui[3], (1) TECNALIA – Parque Tecnológico de Alava, 01510 Miñano/Spain (2) Universidad del Pais Vasco – Escuela de Ingeniería de Bilbao, Alameda de Urquijo s/n , 48013 Bilbao/Spain (3) UMN-PTL - Oak Street SE 200, Minneapolis 55455 2070/United States.

Statistical methods for time series analysis in time and frequency domains are powerful tools for the study of univariate and multivariate stochastic signals, such as those collected by on-line analysers of NOAA aerosols in working scenarios.
This paper explores a statistical approach that uses time and frequency domains in a complementary fashion to analyse data sets, describe, extract and summarize the main characteristics and information contained in stochastic signals, discriminate signals source – background, model and forecast. In addition, applicability of this methodological framework to support quantitative exposure assessments to NOAA, is also discussed.
The stochastic signals were collected during the development of several European and national research projects (e.g. FP7 -Scaffold, EHS-Advance, etc), in industrial scenarios of construction, chemical and foundry & steel, including operations of synthesis and production of nano-TiO_2 and nano-TiO_2 enabled products, as well as handling of nano-TiO_2 in powder form and dispersed in solid matrices.
Measurement data include aerosol time series sampled at source and background, with a set of online analysers (portable and advanced devices), such as CPC 3775, CPC 3007, OPS, Aerotrak 9000 (TSI) and ELPI+ (Dekati). The signals at source were sampled in a fixed point near the worker's breathing zone, and concentration of the background aerosol was determined simultaneously with the source, in a far field representative location. The particle number concentration (total and per measuring channel) and surface area of inhaled particles deposited in the lung, were selected as main metrics. The approaches developed by NIOSH (TiO_2) and nanoGEM were used as sampling strategies

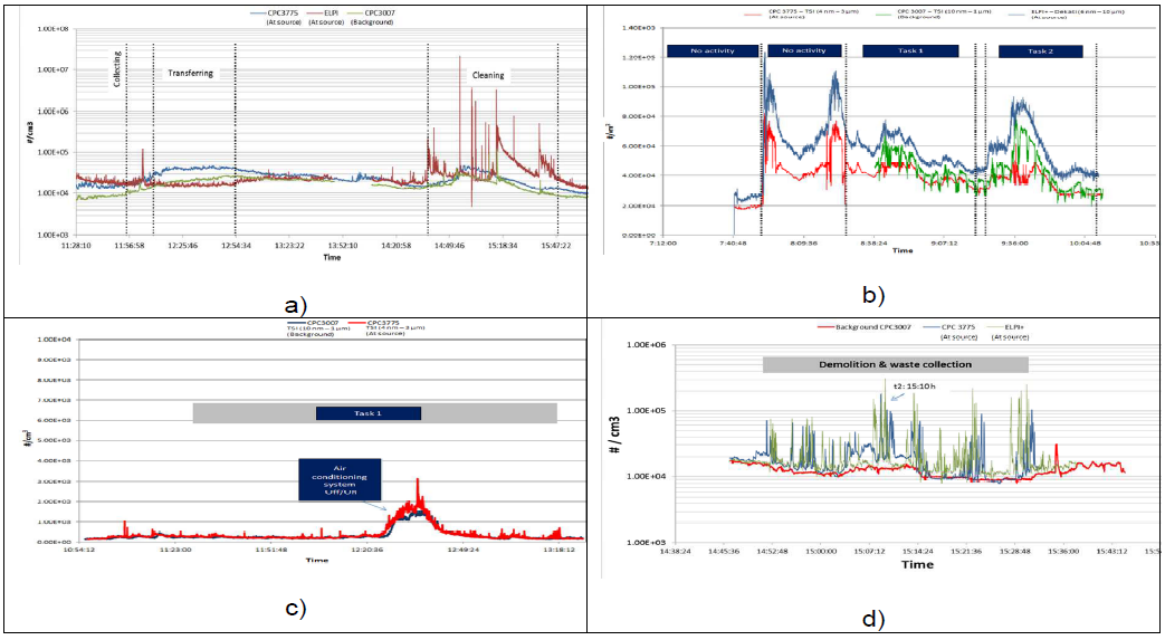

Figure 1 – Figure 1 – Examples of raw signals registered by on-line particle analysers (CPCs-TSI, ELPI+-Dekati) and corresponding to aerosols (at source and background) sampled at different occupational exposure scenarios to nanoTiO_2: a) Synthesis and production of nanoTiO_2, b) Manufacturing of nano-enabled products for steel & foundry, 3) Handling of nano- enabled products containing nanoTiO_2, 4) Demolition of construction products (nanoTiO_2 mortars).

N° O2a-3

EXPOSURE SCENARIO LIBRARIES AS A TOOL FOR EXPOSURE ASSESSMENT

Araceli Sánchez Jiménez[1], Shahzad Rashid[1], Derk Brouwer[2], Wouter Fransman[2], Carlos Fito, George Boulougouris Martie van Tongeren[1]
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Exposure Assessment to engineered nanomaterials (ENMs) is still hampered due to the lack of exposure measurement data and specific exposure models for ENMs
Exposure libraries are a useful tool for exposure assessment of ENMs. Exposure Libraries contain a catalogue of exposure scenarios (ES) and contributing exposure scenarios as described by REACH (Registration, Evaluation, Authorization of Chemicals) where the user can search for a scenario similar to that under investigation and read-across the exposure information. The NANEX project developed an ES data library in MS ACCESS which included 54 occupational and 3 consumer ES, from which 40 occupational ES included measurement data. Exposure measurements for consumer ES was modelled as no data was available. Within the FP7 project MARINA (Managing the Risk of Nanomaterials) an ES library is being further developed based on the NANEX database. Features of the MARINA library ES are that it can be searched by ENM, life cycle stage, process step or contributing scenario. All scenarios include measurement data and the library will be made available online. The library includes ES for occupational exposure, of a range of ENMs (CNT, CeO_2, CrO_3, TiO_2, ZrO_2,nano-Ag, nano-Cu, nano-Fe, QD) and consumer and professional use of various nano enable products (textiles, deodorant, paints, mortar, dental restoration material). The library can be used as a first tier tool within a tiered exposure assessment methodology.
NANEX and MARINA focussed on the inhalation route. Dermal ES and dermal transfer efficiencies are being investigated as part of the FP7 project SUN (Sustainable nanotechnologies) and will be part of an ES Library for dermal exposure.
The FP7 project GUIDENANO will take the concept of ES library a step forward by including an algorithm to quantify the similarity between the library and the scenario under investigation and including data collected within different FP7 projects.
The usefulness of the ES libraries depends on the quality of the contextual exposure information and measurement data. It is key that measurement surveys are carried out in a harmonized way and contextual information is properly recorded
The authors would like to acknowledge the EU Commission FP7 program for funding the three projects mentioned.

N° O2a-4

TOWARDS A STRATEGY FOR ENGINEERED NANOMATERIALS EXPOSURE MONITORING IN THE WORKPLACE: A CASE STUDY.

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With the exponential growth in production and use of engineered nanomaterials (ENMs), a great deal of attention has been paid to potential risks for workers by the scientific community in the field of occupational safety and health. Yet, a critical step in the assessment and management of this emerging risk remains the measurement of occupational exposure to ENMs.
The main objective of this study is to identify the key questions for an approach to the ENMs monitoring at the workplace in order to propose a strategy for the measurement of aerosol particles derived from nanotechnology processes and apply it to a specific case study.
We reviewed the scientific literature on occupational ENMs exposure monitoring and we analyzed the major strategies and measurement techniques of aerosol particparticulate matter in workplace air. Today, different strategies have been put forward worldwide and the discussion is still open on which set of measurement instruments could be employed in an exposure monitoring campaign to better characterize the occupational health effects.
We highlighted some key points for ENMs exposure monitoring, such as the analysis of the production process, the definition of a measurement protocol, the distinction of background airborne particulate matter, the instrument choice and the comprehensive evaluation of exposure, including the integration with toxicological studies on sampled materials.
Moreover, we finalized an experimental study for ENMs monitoring at workplaces, using a multi-disciplinary and multi-parametric approach, through the following tiers of investigation: 1) Preliminary analysis of the exposure scenario, also with information exchange; 2) Pre-observational site walkthrough with qualitative measurements; 3) Pilot studies on laboratory simulations of processes, useful to the exposure scenario identification. 4) Measurement campaign at the workplaces with following in-bulk sample analysis.
A tiered approach has been suitably applied, also in order to balance costs and benefits, and the outputs of this study have generated useful data for the characterization of the exposure scenario in the specific production process. Furthermore, a multi-parametric approach has contributed to the distinction of background aerosol coming from other sources in the same workplace. Finally, the exchange of information has helped us gain a broader understanding of risk evaluation and enhanced the cooperation between public research and enterprises at national level.
The application of this strategy to the occupational risk assessment process and the integration of results into the existing databases require an improvement of our research efforts towards the harmonization of approaches in the framework of ENMs exposure monitoring strategies at international level.

N° O2a-5

BIOMONITORING TO NANOPARTICLE EXPOSURE: APPROACHES FOR THE DEVELOPMENT OF INDICATORS OF EXPOSURE AND EFFECT

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The use of engineered nanoparticles (NP) is more and more widespread in various industrial sectors, although their toxicity is not fully elucidated and exposure levels are poorly documented. Exposure levels might be particularly elevated in work places where NP can be handled in large amounts and as powders. The inhalation route of exposure seems notably worrying due to the known adverse effects of ultrafine air pollution and of asbestos as well. Along with the application of the precaution principle, assessment of exposure must be addressed and external measurement have to be completed by biomonitoring which is the only way to take into account several parameters at the individual level, such as the use of protective devices or the ventilation mode for instance. However, no recommendation for biomonitoring to NP is currently available.

The Medical Biology Laboratory of CEA Grenoble, entirely devoted to occupational health in close link with occupational physicians, is working on different approaches to develop indicators of exposure and effect useful in the field of occupational health. Regarding indicators of exposure, new tools are set up to assess potentially inhaled NP in non-invasive respiratory sampling such as nasal sampling and exhaled breath condensate (EBC). Different analytical methods are used to detect and characterize NP, such as ICP-MS for elemental composition, dynamic light scattering for size distribution and electronic microscopy coupled to energy-dispersive X-ray for physicochemical characterization. Respecting indicators of effect, a methodology has been developed to assess a panel of 29 cytokines in exhaled breath condensate as the reflection of potential respiratory inflammation due to NP exposure. The panel of cytokines has been determined in a population of non-exposed subjects in order to get a reference panel to be compared to future analyses in exposed workers. Second, a collaboration between the LBM and the EDyp team has led to the characterization of the proteome of EBC. 99 proteins of pulmonary origin have been identified in a pool of EBC (10 healthy donors) using LC-MS/MS after concentration of the EBC samples using lyophilization, SDS-PAGE separation and trypsin digestion. The next step will also consist in applying the developed method to the analysis of the proteome of EBC from exposed workers so as to identify potential proteins modulated by NP exposure and therefore potential indicators of exposure.

The ongoing projects should help the development of new tools to biomonitor individual NP exposure and potential early impacts on health. Innovative techniques such as field-flow fractionation coupled to ICP-MS and single particle-ICPMS are being explored for the measurement of NP in biological media. These tools are directly dedicated to occupational physicians to help them in the identification of exposure situations, and in the management of worker health.

N° O2b-1

OCCUPATIONAL EXPOSURE TO NANO-TiO_2 IN THE LIFE CYCLE STEPS OF NEW DEPOLLUTANT MORTARS USED IN CONSTRUCTION

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In the recent years the construction sector has incorporated new materials based on nanotechnology. This has raised issues about occupational exposure. The present work is focused on the measurement of workers exposure to nano-TiO_2 in the life cycle steps of depollutant mortars. It has been done in the framework of the SCAFFOLD project, which aims at the management of potential risks arising from the use of manufactured nanomaterials in construction.

The measurements include the following processes: depollutant mortar fabrication, its application in walls, machining of materials during use and finally, demolition. Additionally, the manufacturing process of nanoTiO_2 has been considered. Measurements have been performed at pilot and industrial scale in similar conditions to those of real practice.

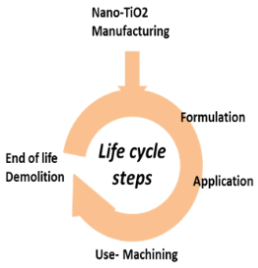
The exposure assessment strategy followed NIOSH (Bulletin 63) (NIOSH, 2011). Samples at the personal breathing zone have been collected for off-line ICP-MS and SEM/EDX analysis. Simultaneously, the aerosols released in the activities have been characterized using on-line devices following the tiered approach established by Asbach et al. (2012).Occupational exposure limits used for nano-TiO_2 are 0.3 mg/cm3 (NIOSH) and 0.1 mg/cm3 (Scaffold project).

Main findings can be summarized as follows:

- It was found a high release of particles above the background in several tasks as expected due to the nature of the activities performed. The maximum concentration was measured during drilling (mean total particle concentration up to 2.71E+04 particles/cm^3) or during mixing powder materials (3.32E+04 particles/cm^3). However, considering data on total particle concentration released, no differences have been observed when tasks have been performed using conventional materials in the sector (control) and when using materials doped with nano-objects.
- The occupational exposure to nano-TiO_2 is below 0.3 mg/m^3 for all measured scenarios. The highest concentrations were measured during the cleaning task (in the nano-TiO_2 manufacturing process) and during the application (spraying) of depollutant coatings on a wall.

These findings contribute to clarify the discussion about whether the incorporation of nano-objects to the construction industry may originate an increase on the risks for workers in this sector.

Figure 1. Main measurement results



	Particle Released (1)		Occupational exposure to nano-TiO2		
	Total particle concentration (mean)		sampling time	8 h TWA (2)	SEM (3)
Life cycle step	CPC 3007 (#/cm3)	OPS (#/cm3)	[min]	(mg/m3)	
TiO2 manufacturing					
Cleaning/maintenance	0	138	62	0.048	Yes
Depollutant mortar formulation	3.32E+04	5.44E+03	59	0.073	Yes
Depollutant mortar application	6.42E+03	226	24	0.055	-
Spraying sol-gel	1.65E+04	1.02E+03	13	0.195	Yes
Drilling Depollutant mortar	2.71E+04	-	7	-	-
Demolition	6.66E+03	0	37	0.009	-

(1) It is showed maxium values measured in the process
(2) It has been considered the worst case, considering that the task is peformed during the 8 h/day, with the exception of the cleaning/maintenance task were the semanal exposure has been considered
(3) Evidence of the nano-object on the samples (Yes/No)

N° O2b-2

QUANTITATIVE CHARACTERIZATION OF AIRBORNE PARTICULATE RELEASE DURING SPRAY-CAN AND SPRAY-GUN APPLICATION OF NANOPARTICLE-DOPED COATINGS

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The coating industry processes numerous materials within their products, which are covered by the definition of nanomaterials according to ISO/TS 80004-1:2010 and the re-commendation of the European Commission (2011). In fact, there are several potential release scenarios for nano-objects during processing and use of coating products (Brouwer, 2010). A comparison between potential release scenarios (e.g. Brouwer, 2010) and performed release studies (e.g. Kuhlbusch et al. 2011, Froggett et al. 2014, Losert et al. 2014) shows that until now only less information are available for spray application of nanoparticle-doped coatings.

In order to characterize particulate emissions during spray-can and spray-gun application, a simple spray channel (Göhler et al. 2014) was developed. To ensure occupational and instrumental safety, the spray channel was implemented in a special designed experimental setup. The spray-aerosol characterisation was performed according to the systematic approach of Göhler et al. (2013), i.e. among others macroscopic spray process characteristics were analysed in addition to the particulate release. An Engine Exhaust Particle Sizer, an Aerodynamic Particle Sizer and a Condensation Particle Counter were operated for the determination of number-weighted particle size distributions and particle number concentrations from a few nanometres up to several micrometres. Particle nature analyses were performed on electrostatically precipitated spray aerosol particles by means of scanning electron microscopy, transmission electron microscopy and energy-dispersive X-ray spectroscopy.

The authors will discuss experimental details. Release data will be given for four types of coatings doped with three types of nanoparticle additives that were aerosolized by two kinds of spray cans a manual gravity spray gun.

Figure 1. Example of an analyzed standard propellant spray can.

Acknowledgment.

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N° O2b-3

FIRST DEVELOPMENT TO MODEL AEROSOL EMISSION FROM ENGINEERING MATERIALS SUBJECTED TO MECHANICAL STRESSES

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Many studies have shown that when the surface of traditional and nanostructured materials are subjected to mechanical stress, aerosolization of wear particles can occur [1]. Such an aerosol comprises of nano and micro particles which are prone to human interactions, either through their inhalation or dermal contact.
To expand our knowledge on the release mechanisms of these particles, an attempt has been made to develop a mathematical model. Thanks to prior studies [2, 3, 4], this model considers an original and multidisciplinary approach that links different constitutive equations in Tribology, Material and Aerosol physics.
Among other hypotheses, a Rossin-Rammler distribution is used to approximate the size distribution of generated abrasion wear particles in nanosize range ($\leq 10^{-7}$ m). It is normally used to fit the size distribution of microparticles, generated during the fragmentation of rocks. Furthermore, the generated wear particles are hypothesized to be spherical in shape and their aerosolization probability depends upon its size, density, environmental conditions and the net mechanical force input.
The experimental data showed varying levels of generated aerosol particles which primarily depends upon the type of material getting abraded i.e. it can be high (e.g. in case of brick and ceramic) as well as contrastingly low (e.g. in case of steel). This variability, observed in the experimental data, is also predicted by the model. A sensitivity analysis was also conducted to determine the relative importance of various key parameters (like material, process and environment parameters).

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N° O2c-1

EXPOSURE TO AIRBORNE NANO-SIZED PARTICLES FROM CERAMIC MILLING PROCESSES

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Evidence that workers in the ceramic industry are exposed to harmful airborne particles has been increasing, especially during ceramic manufacture in furnaces [1, 2]. However, much less is known about the emission of ultrafine particles (< 100 nm in diameter) from mechanical processes such as dry milling. The present work aims to identify and quantify particle emissions (with special interest in airborne nanoparticles; Dp < 50 nm) from dry milling activities with a pendulum mill under various conditions: (i) two different feed materials (red clay and red-body stoneware) and (ii) three different energy settings (high, intermediate and low). Particle number, mass concentrations, alveolar lung deposited surface area concentration and size distributions in the 10 - 20000 nm size range were simultaneously monitored in the worker breathing zone and in outdoor air by using a switch-valve system connected to the particle monitoring instrumentation (SMPS, NanoScan, OPS, Grimm optical particle counter 1108, DiscMini and CPC). Samples were also collected to characterize the particles and determine their elemental composition by transmission electron microscopy (TEM) coupled with energy-dispersive X-ray spectroscopy.

The results evidenced that nanoparticles were generated and emitted into workplace air during dry milling, despite the fact that pendulum mills are used to mill materials to much coarser particle diameters (~40 µm). Particle emission behaviours were different for both feed materials and were strongly dependent on their composition and the selected energy settings.

Overall, average particle number concentrations during red clay and red-body stoneware milling under the high energy setting were 2 and 5 times higher than background concentrations, respectively as shown in Figure 1. The highest values of particle number concentrations were obtained by using high energy settings, especially during red-body stoneware milling, reaching a maximum of 1.3 x 10^5 particles·cm^{-3} with mean particle diameters of 37 nm. During red clay milling under the high energy setting, PM_1, $PM_{2.5}$ and PM_{10} concentrations were found to be 2.1, 1.9 and 1.3 times higher than those obtained with red-body stoneware milling.

Furthermore, during red-body stoneware milling, a proportion of 99.6% of the new particle emissions (by subtracting the background from the total particle number concentration) was in the nucleation mode (<30 nm in diameter [3]), while during red clay milling 86% of the particles was > 100 nm, with 24% of the particles in the 100-200 nm size range (Figure 1).

The results from this study evidence the risk of worker exposure to ultrafine and nanoparticles during ceramic milling processes and raise a need to develop mitigation strategies.

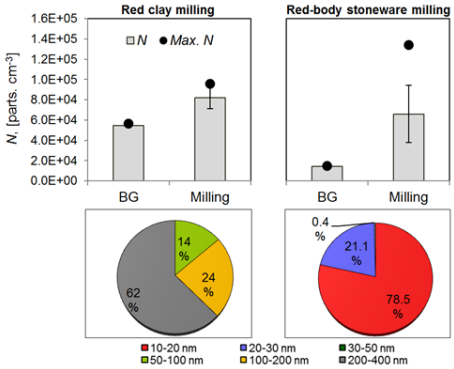

Figure 1. Contribution of particle number concentration (%) in different size ranges. BG: background concentrations.

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N° O2c-2

OCCUPATIONAL EXPOSURE ASSESSMENT DURING HIGH VOLUME SYNTHESIS AND SUBSEQUENT HANDLING OF MULTI-WALLED CARBON NANOTUBES.

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Introduction: Carbon Nanotubes (CNTs) are increasingly produced. To date, a limited number of CNT exposure assessments were identified during the high volume synthesis and subsequent handling. The goals of this study were to assess personal exposure during the high volume synthesis and subsequent handling of Multi-Walled Carbon Nanotubes (MWCNTs), by a specific marker for CNT mass, and to link assessed exposure levels to the performed activities.
Method: 58 personal full-shift filter-based samples (25 mm IOM inhalable fraction) were collected at a large company producing MWCNTs during synthesis and handling of high volume MWCNT, R&D activities and in offices. Morphology and particle size distribution of the particles were assessed by scanning electron microscopy (SEM) in combination with energy dispersive x-ray spectroscopy (EDX). Mass concentrations were obtained by carbon analysis, separating elemental carbon into three stages (according to different oxidized temperatures), distinguishing between MWCNTs and atmospheric particulate matter. Linear mixed-effects models were used to study associations between exposure and location or activities with worker included as a random effect.
Results: MWCNTs were present as large conglomerates ranging between 500 nm – 10 µm. Exposure levels of MWCNTs were comparable in the production facility during the full scale synthesis of MWCNTs (GM (95% LCL-95% UCL)): 40.82 µg/m^3 (18.89- 88.20)) and during a period of further handling of MWCNTs: 43.17 µg/m^3 (21.78- 85.59)). In the R&D facility and an office, exposure levels of MWCNTs were lower: 4.52 µg/m^3 (1.79 – 11.47) and 7.14 µg/m^3 (1.83 – 27.85), respectively. Bagging, maintenance of the reactor, cleaning and grinding were associated with higher exposure levels in the production facility, whereas increased exposure levels in the R&D facility was related to handling MWCNTs powder.
Discussion and conclusion: This study shows that high volume production is associated with higher exposure levels than R&D activities, using EC as a specific marker for CNT exposure. Several high exposure risk activities were identified. The exposure levels will be used in a cross-sectional study of biological markers in the workers of this facility.

N° O2c-3

ASSESSMENT OF EXPOSURE TO ENGINEERED NANOMATERIALS DURING MANUFACTURING AND DOWNSTREAM USE OF PIGMENTS, INKS AND PAINTS

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We present the methodology and results from an exposure assessment of engineered nanomaterials (ENM) produced and used in the pigments, fillers and inks industry {cobalt aluminate spinel ($CoAl_2O_3$); quantum dots with a cadmium selenide core and a zinc sulphide coating (CdSe/ZnS); silver (Ag); zinc oxide (ZnO); titanium dioxide (TiO_2) and a nano-Ag based ink}. This work is part of a larger FP7 project (NANOMICEX) seeking to synthesise, characterise and commercialise low toxicity/high performance nano-pigments, inks and paints by surface modification. Twelve (five industrial, four consultancy and three academic) partners collaborated in NANOMICEX.
A tiered approach was used in the exposure assessment. The aim of Tier 1 was to identify and rank relevant exposure scenarios (ES). All five companies hosted Tier 1 scoping visits at their facilities. Six processes were observed or monitored with portable instruments (condensation particle counter, CPC; optical particle sizer (OPS) and DustTrac DRX). Contextual data were collected on the processing techniques, the material streams, scale of operation, production equipment and operating conditions (OC), work patterns (frequency, duration, number of operators, level of manual intervention) and risk management measures and used to develop the ES. These companies were one pilot and one laboratory scale manufacturer of ENM, one laboratory scale producer of ENM dispersions, and two downstream users of ENM as powder and colloidal dispersions (in paint formulation) and as colloidal dispersions (in inkjet printing).
Three of the five companies, with potentially exposure-significant ES, were selected for the Tier 2 measurement surveys. This involved monitoring particle concentration and size distributions prior to (background) and during ENM manufacture and use at work area locations. Real time measurements were taken using multiple direct reading instruments {CPC, OPS, fast mobility particle sizer (FMPS), and aerodynamic particle sizer (APS)}. Filter and surface tape samples were analysed by Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDXS) for morphological and compositional data on airborne and settled particles respectively.
Eight activities with a potential to release nanoparticles were identified across the ES, as in the observed processes or after their 'extrapolation' to an industrial scale. These are synthesis via a pyrolysis reaction; harvesting/isolating synthesised or functionalised ENM; drying an ENM paste to a powder; milling an ENM powder; charging a process with powdered ENM; packaging and maintenance work on production plant and on ventilation systems. The measurement results for NANOMICEX will be reported in the conference presentation.
Acknowledgement: funding from EC under the 7th Framework Programme (Grant Agreement number 280713)

N° O2c-4

EXPOSURE ASSESSMENT TO NOAA DURING MIXING OF NANOMATERIALS POWDERS

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Currently, the number of investigations of emission of NOAA (nano-objects, agglomerates & aggregates) during work performed with nanomaterials is still limited and there is lack of generally accepted principles of assessment of exposure to NOAA with respect to the limit values. One option for estimate the likelihood to exposure to NOAA is "decision logic" described in paper [1]. Generally, there are three main steps to assess the exposure: (1) determination the ratio of average concentrations particles obtained with direct-reading instruments during process with nanomaterials compare to background/no activity, (2) confirmation of presence of NOAA in the air from the TEM and EDX analysis of air sample taking simultaneously with direct-reading measurements, (3) observation possibility of presence of another sources of particles than that from the processes with nanomaterials. The aim of investigations was to confirm or exclude the likelihood of exposure to NOAA during mixing commercially available 9 kinds of nanomaterials containing different types of nano-objects, namely 5 types of nanoparticles (cooper, zinc iron oxide, zirconium oxide, zirconium oxide Ca stabilized, zirconium oxide Y stabilized), 2 types of nanoplates (nanoclays: nanomer PGV, nanomer I.34 TCN when measured were done for dry and no dry powders of nanomer I.34 TCN) and 2 types of nanotubes (halloysite nanoclay, MWCNT 30-50nm). Studies have been done without another significant sources of particles in addition to those associated with the mixing of nanomaterials powders. NOAA parameters were obtained with direct-reading instruments, as SMPS (number concentration and size distribution of particles 16-661nm), P-Trak (number concentration of particles 20-1000nm) and Aero-Trak (surface concentration of particles 10-1000nm). During processes of mixing nanomaterials powders air samples with NAS were taking for future analysis with TEM or SEM and EDX.

For all processes of mixing nanomaterials powders were found statistically significant increase of particles concentrations measured with SMPS, P-Trak and Aero-Trak during activities compared to that obtained for background/no activity. TEM or SEM and EDX analysis confirmed that NOAA from mixing nanomaterials powders presented in the air. Furthermore, for all studied processes of mixing, the results of size distribution confirmed increase of concentrations of nano-sized particles compare to concentrations obtained for background/no activity.

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This study has been prepared within NANODEVICE project (number FFP7 211464) and CIOP-PIB project (No.2.Z.04).

N° O2c-5

STRATEGY FOR THE LOWERING AND THE ASSESSMENT OF EXPOSURE TO NANOPARTICLES AT WORKSPACE
CASE OF STUDY CONCERNING THE POTENTIAL EMISSION OF NANOPARTICLES OF LEAD IN AN EPITAXY LABORATORY

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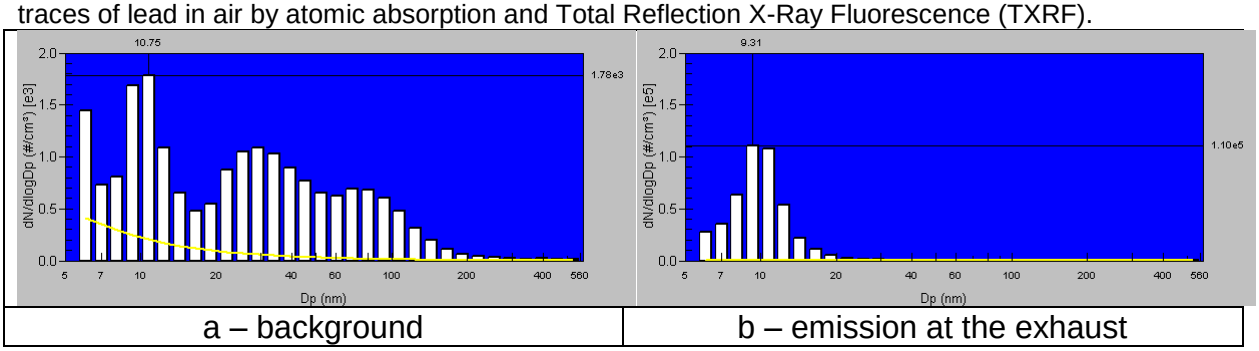
The CEA - NanoSafety Plateform is developing various research topics for health and safety, environment and nanoparticles exposure, in professional activities. The optimisation of containment for lowering the exposition, then the assessment of exposure to nanoparticles is a strategy for safety improvement at workplace and workspace. The lowering step consists in an optimisation of dynamic and static containment at workplace and/or workspace. Generally, the exposure risk due to the presence of nanoparticles substances does not allow modifying the parameters of containment at workplace and/or workspace. Therefore, gaseous or nanoparticulate tracers are used to evaluate performances of containment. Using a tracer allows to modify safely the parameters of the dynamic containment (ventilation, flow, speed) and to study several configurations of static containment. Moreover, a tracer allows simulating accidental or incidental situation. As a result, a security procedure can be written more easily in order to manage this type of situation. The step of measurement and characterization of aerosols can therefore be used to assess the exposition at workplace and workspace.
The case of study, aim of this paper, concerns the potential emission of nanoparticles of lead in an epitaxy laboratory. The use of helium tracer to evaluate the performance of containment is firstly studied. The efficiency of two extraction systems, located at the exhaust of an epitaxy furnace, is evaluated. In addition, the influence of a refractory component, placed at the exhaust of the furnace, to canalise the lead vapour is studied.
Secondly, the assessment of exposure is characterised in accordance with the French guide “recommendations for characterizing potential emissions and exposure to aerosols released from nanomaterials in workplace operations”. These recommendations should be proposed to international regulation. The characterization of aerosols includes measurements of the concentration using condensation particle counters and measurements of the size distribution based on electrical mobility of particles – see figure.
Thirdly the aerosols are sampled, on several places, using collection membranes to try to detect traces of lead in air by atomic absorption and Total Reflection X-Ray Fluorescence (TXRF).



a – background	b – emission at the exhaust

Distribution in background (a) and during an emission at the backside of the exhaust of the epitaxy furnace (b)

N° PL3

MEASUREMENT AND FILTRATION OF AIR/LIQUID/SURFACE-BORNE NANOPARTICLES IN SUPPORT OF SUSTAINABLE NANOTECHNOLOGY

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Nanomaterials/nanoparticles (NPs) are produced at a rapid pace in recent years. There is increasing concern with the occupational health and safety of NPs in the workplace. Another concern deals with the implications of NPs, both engineered NPs and environmental NPs from engine emissions, on the environment and living systems. Environmental, health and safety studies of NPs (Nano-EHS) are therefore needed to support sustainable nanotechnology. At the Center for Filtration Research (CFR), consisting of 15 filter manufacturers and end users plus NIOSH as an affiliated member, we have conducted research on Nano-EHS, involving physico-chemical characterization, exposure and toxicity, and abatement by filtration of NPs. Since NPs exist in the air-borne, liquid-borne and surface-borne phases, we have developed instruments and measurement techniques to detect NPs in all three dispersed phases, which are also necessary for the life-cycle assessment of NPs in the environment. Recent advances have focused on real-time detection and characterization of nano-agglomerates and of NPs surface area. Our research has led to several government and industry standards on NPs, for both particle size and concentration. Filtration is one of the principal means to mitigate workers' exposure to the accidental release of NPs. Integrative approach is used, which combines knowledge from several interdisciplinary fields for filtration research. Several topics of nanoscale filtration performed in CFR will be presented. They include sampling and filtration of airborne CNTs, liquid filtration of sub<10 nm quantum dots, and $PM_{2.5}$ particulate loading on filter surfaces.

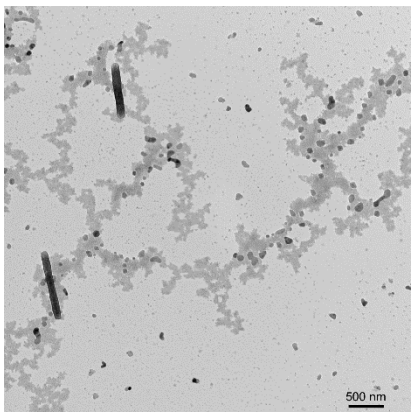
N° O3a-1

TOWARDS AN INDICATOR OF NANOMATERIAL DEPOSITION IN THE HUMAN LUNG

Dimitrios Bitounis[1], Cyril Guibert[2], Valérie Forest[1], Delphine Boudard[1,2], Jérémie Pourchez[1], Jean Michel Vergnon[1,2], Michèle Cottier[1,2](1) LINA EA 4624 - 15, rue Ambroise Paré, Jean Monnet Medical University, Saint-Etienne, France(2) Pneumology and Histology-Cytology Departments – Av. Albert Raimond, Central University Hospital, Saint-Etienne, France.

Various engineered nanomaterials (ENM) have already been incorporated to large-scale industrial processes while their toxicological profiling is still under way. In light of this fact, workplace exposure to ENM merits increased scrutinization.
Bronchial washings (BW) are diagnostic procedures during which instilled saline solution recovers the components from the epithelial surface to the larger airways of the lower respiratory system. It could also be able to provide insight to deposited particles in the lungs, i.e. the main route of human exposure to airborne particles. The aim of this work is to identify in BW an indicator of deposited engineered metal and metal oxide particles to the lungs. To this end, we have optimized the extraction of sufficiently dense nano-sized particles from BW and have subsequently performed their physicochemical characterization and semi-quantitative analysis. To this day, 60 patients have been included in the study all of whom presented symptoms of infiltrative pulmonary syndromes when admitted in Centre Hospitalier Universitaire of Saint-Etienne, France.
BW was centrifuged on top of glycerol–NaOH(aq) cushions. Most of the proteins, glycoproteins and small sub-cellular debris were thus left in the supernatant, while sufficiently dense or large particles were forced into the glycerol compartment. The pellet was then reconstituted with NaOH(aq), sonicated and measured by dynamic light scattering (DLS). The elemental compositions of both the pellet and the supernatant were measured by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES). The samples for which DLS and ICP-AES corroborated a possible particulate load were observed under transmission electron microscopy (TEM). Presented here is the nanoparticle extraction protocol, the analysis of samples from the first 9 patients and how this data could function as an alert indicator for human exposure to ENM.
This work is both original and urgent, as it is the biggest clinical study specifically designed to detect and analyze ENM in human biological samples, at a point in time when workplace exposure to ENM is expected to get higher.

Transmission electron microscopy image of



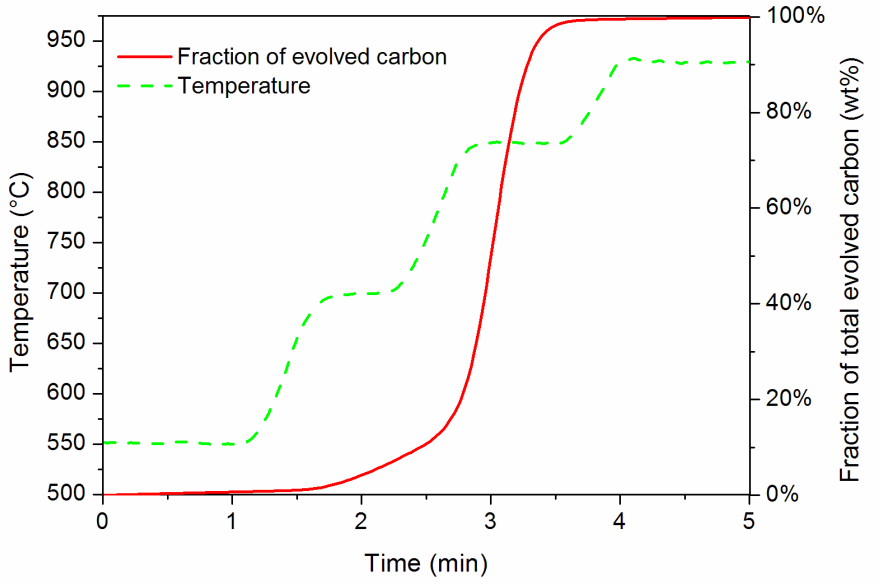
nanoparticles and nanofibers deposited in the lungs of a patient

N° O3a-2

IDENTIFICATION OF CARBON NANOTUBES BY THERMAL-OPTICAL ANALYSIS

B. Faure[1], P. Babbar[1], A. Guiot[1], S. Artous[1], P. Tiquet[1], S. Clavaguera[1], S. Derrough[1], J.-F. Damlencourt[1] (1) Univ. Grenoble Alpes, F-38 000 Grenoble, France. Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), F-38 054 Grenoble, France.

The unique properties of carbon nanotubes (CNTs), *e.g.* their high mechanical stiffness and strength, tunable electronic properties, high electrical and thermal conductivities and low density, make them valuable in many applications. As the quantities of CNTs produced are expected to rise, concern has been expressed about the potential health effects of CNTs for workers involved in their handling. Exposure by inhalation is considered the most probable route, and the presence of CNTs in the pulmonary tract is particularly concerning due to their size and fibrous morphology.
The toxicity of CNTs depend strongly on their properties, such as tube length, diameter and stiffness, number of walls, surface functionalization and metal impurities, which cannot be assessed by a single metric. Nevertheless mass concentration has been used in toxicological studies to provide occupational exposure limits. Fast and simple optical or thermal methods are available to quantify carbon particles by mass, *e.g.* aethalometers, thermogravimetry or thermal-optical analysis. However these methods do not selectively quantify CNTs and need to be supported by electron microscopy observations, which are labor-intensive and hardly applicable for widespread monitoring.
The thermal-optical analysis relies on the quantification of carbon dioxide evolving from a carbonaceous sample upon heating in a controlled atmosphere. Although this technique is routinely used for the quantification of soot particles, little is known on the possibility to use the thermal degradation profile as a fingerprint to distinguish CNT products from other carbonaceous particles. Several commercial single- and multi-wall CNTs have been analyzed to identify the main parameters (*e.g.* tube diameter, metal impurities) affecting their thermal stability. The ability of the technique to distinguish CNTs mixed with background particles from ambient air has been evaluated. Finally, some guidelines on the design of the thermal evolution protocol will be presented.



Thermal evolution of carbon from a Nanocyl 7000 CNT sample in a He/O_2 atmosphere.

This work was supported by the LabEx SERENADE (Laboratory of Excellence for Safe(r) Ecodesign Research and Education applied to NAnomaterial DEvelopment).

N° O3a-3

EXPERIMENTAL CHALLENGES FOR THE DETECTION OF NANOPARTICLES IN FOOD AND COSMETIC PRODUCTS

R. R. Retamal Marín[1], F. Babick[1], M. Stintz[1], (1) Technische Universität Dresden, Research Group Mechanical Process Engineering, Dresden/Germany

Understanding the world of nanoparticles, especially their interactions with environment, begins at first with their correct detection and successive quantification. To achieve this purpose, one needs to perform correctly developed standard operating procedures (SOPs). Furthermore, the study of nanoparticles frequently requires their detection and characterisation in complex media (e. g. in cosmetic formulations).
The present study focusses on synthetic amorphous silica (SAS) based on its use in a wide range of consumer products including cosmetics (hydrated silica) and foods (E551) in form of powder, dilute liquid suspensions, emulsions or pastes. SAS-particles consist of fractal DLCA-like aggregates with constituent particles in the range < 100 nm and thus belong to the class of nanomaterials. Due to the current legislation and the interests of consumer protection it is highly necessary to evaluate the state of dispersion of SAS particles, i. e. the size distribution of agglomerates and aggregates in colloid range.
Complex media are particle systems with different dispersed (= particulate) phases. Examples may be aqueous emulsions with an oily droplet phase, several solid phases and nanosized micellar structures of surfactants and polymers. Obviously, it is not trivial to detect and quantify nanoparticles ($\leq$ 100 nm) in such systems. Further problems arise from the fact that the dispersed phases typically cover a wide range of particles sizes (of a few nanometres up to even millimetre). The accurate determination of particle size distribution – in particular the quantification of the nanoparticle content – therefore requires appropriate preparation techniques and adopted measurement procedures.
This paper discusses the development of preparation techniques for the adequate characterisation of SAS-particles in complex media. These techniques combine the defined separation of coarse micrometre particles with the selective separation of SAS from oil droplets. The effectiveness of these procedures is discussed for hydrophobic and hydrophilic SAS products and for different SAS sizes, which depends on a) the “dispersion history” and b) the agglomerate/aggregate strength of the respective SAS products.

N° O3a-4

DETECTION OF CARBON NANOTUBES AFTER AN ABRASION EXPERIMENT

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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 an epoxy nanocomposite when an abrasion process is applied (Schlagenhauf *et al*. 2012). This result corroborates the need of a proper risk assessment as it is known that inhaled CNTs can be toxic (Ma-Hock *et al.* 2009). Therefore, it is crucial to develop a measurement method to detect and quantify the released CNTs in order to determine the exposure.

After abrading particles from an epoxy/CNT nanocomposite, three different kinds of CNTs are present in the produced dust: CNTs that are completely embedded in the polymer matrix, CNTs that are protruding from polymer particles, and free standing CNTs. When inhaled, the free standing and protruding CNTs may directly contact the lung cells, thus causing more severe toxic impact than the still embedded CNTs. Therefore it is crucial to develop a method to differentiate the different kinds of CNTs.

A method is going to be presented where CNTs are marked with metal ions by adsorption on the surface. After abrasion of a composite with the labeled CNTs, particles can be collected and immersed in an acidic solution in order to desorb the ions from the exposed CNTs while the ions on the embedded CNTs remain bound. By determination of the ion concentration by ICP-MS, the quantity of the free standing and protruding CNTs can be estimated.

The method can be refined by the collection of particles in different size ranges. While the collection of all abraded particles gives an estimation of the impact on the environment, the collection of inhalable particles characterizes the release correlated to the health impact on people who process the material. Further, the collection of particles with a diameter below 100 nm could be used to detect free standing CNTs.

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Ma-Hock L, Treumann S, Strauss V, Brill S, Luizi F, Mertler M, Wiench K, Gamer AO, van Ravenzwaay B, Landsiedel R, Toxicological Sciences, 2009, 112, p.468-481

N° O3a-5

THE USE OF SMALL-ANGLE X-RAY SCATTERING FOR THE CHARACTERIZATION OF NANOPARTICLES IN BIOLOGICAL MATRICES

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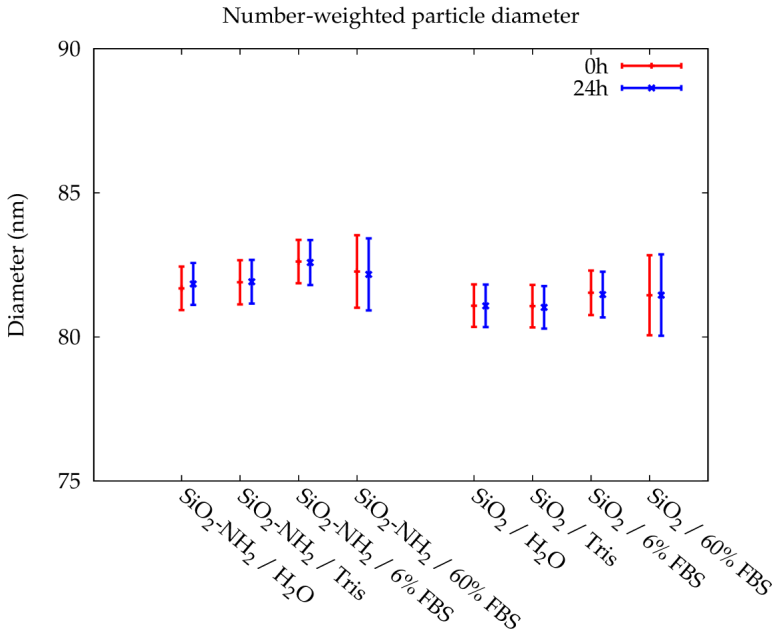
The increasing production of engineered nanomaterials raises concerns due to their potential hazards, which is in connection with their physical and chemical properties. The characterization of nanoparticles (NPs) is therefore in the forefront of metrology research, which also aims at the better understanding of the interaction of NPs with biological matrices by developing traceable methods. A traceable measurement of NPs in biological media is challenged by the presence of many components in the sample, and also by the fact that the biological fluid can modify the characteristics of NPs due to the association of biopolymers to their surface.
In this work, small-angle X-ray scattering (SAXS) was used for the traceable characterization of plain and aminated silica NPs in different biological media. The results have proven that SAXS can be used for accurate determination of the mean diameter of NPs even in a media containing 60% serum, which cannot be achieved using light scattering methods. Moreover, SAXS was also used for the characterization of the protein corona formed around NPs when dispersed in serum. The latter is of great importance, as the absorbed proteins determine the in vivo biodistribution of NPs. Despite the extensive research on the field, accurate structural characterization of the hard protein corona formed around NPs has not yet been reported in the literature. By utilizing contrast variation SAXS, i.e. the variation of the electron density contrast between the particles and the suspending medium by changing the chemical composition of the latter, we can provide an accurate description of the protein layer around silica and polystyrene NPs.
This work was funded by the European Metrology Research Programme (EMRP) under the Joint Research Project NEW03 (NanoChOp). The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union.



Number-weighted diameters of plain and aminated silica particles in different media as revealed by SAXS. (Bars represents the standard uncertainty)

N° O3a-6

PROPERTIES OF NANOPARTICLES AFFECTING SIMULATION OF FIBROUS GAS FILTER PERFORMANCE

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Computational Fluid Dynamics (CFD) software now widely available allows detailed simulation of the flow of gases through fibrous filter media. When the pattern of gas velocity vectors in the interstices between fibers has been established, a simulated particle of any desired size can be “injected” into the entering gas stream, and its path under the influence of aerodynamic drag, Brownian motion, electrostatic forces and thermal gradients traced, until the particle either collides with a fiber, or passes through the entire filter medium. Successive simulated injection of many particles at random locations in the entering stream allows an average probability of capture to be calculated. If the particles can be assumed to adhere permanently to fibers after a collision, the average thus determined will represent the capture probability, or “efficiency” for that particle type and size of particle.

Many particle properties must be available as parameters for the equations defining aerodynamic drag, electrical and thermal mobility, and Brownian velocity. Particle morphology, size, density, Knudsen Number, charge, and dielectric constant are examples of influential properties. These properties have been characterized and measured in many studies for micrometer-scale particles, but less so for nanoparticles.

A particle which collides with a fiber does not necessarily remain permanently attached to that fiber. Under some conditions, a particle will bounce off the fiber, re-entering the gas stream with some velocity and direction dependent its shape, its velocity and angle of approach to the fiber surface, and the elastic properties of the particle-fiber combination. If the particle does not bounce, it may still be bound to the fiber surface weakly enough to be removed by the gas flow past the fiber, or by vibration.

A particle retained on the fiber surface becomes a potential object for collisions by later arrivals of its own kind. Indeed, the formation of fiber-like chains of captured particles – dendrites –has been observed in filter media. Thus both particle-fiber and particle-particle bounce and adhesion properties are needed for a complete simulation of particle filtration. This is especially important in simulating the “loading” of filter media, which can enable prediction of the rise in pressure drop across a filter medium when some distribution of aerosol particles is fed to it over an extended period of time.

Accurate values for all properties affecting particle dynamics are needed, not only for predicting particle capture in actual service, but also to validate models for media geometries and computational procedures used in CFD.

We present a survey of existing literature on the properties influencing the effects listed above, with emphasis on nanoparticles where available. In some cases, effects which are significant for particles with micrometer dimensions are trivial for nanoparticles; in some cases, the reverse is true. We discuss the reliability of extrapolation of property values from micrometer-scale studies to nanoparticles, where nanoparticle data are not available.

N° O3a-7

INVESTIGATION OF THE LIFE CYCLE OF TITANIA NPS USING RADIOLABELING TECHNIQUES FOR HIGHLY SENSITIVE NP DETECTION

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Production and application of TiO_2-containing nanocomposites such as functional surface coatings have significantly increased in recent years. These coatings are used in a wide field of applications ranging from self-cleaning and scratch resistant surfaces to biocidal coatings. Therefore, knowledge about potential nanoparticle (NP) release due to aging or abrasion of these coatings is essential for safe application of these materials.
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 readily quantified.
The joint research project NanoTrack used model surface coatings in an acrylate-based formulation containing TiO_2 NPs (d = 21 nm, P25, Evonik Industries). Coatings were produced by application of 25 µm thick nanocomposite layers on a substrate followed by curing and later weathered under standard laboratory test conditions. Due to the low resistivity of this model system, the organic matrix of the surface coating was severely degraded and NPs 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. For industrial nanocomposites (realistic case), the same weathering procedure resulted in release of only small amounts of TiO_2-NPs. Nevertheless, radioactivity detection methods proved this release.
Current studies on the environmental fate and effects of nanoparticles are limited by the inability to detect and quantify nanoparticles in complex environmental test systems and radiolabeling nanoparticles may provide a solution to this limitation. Isotopic labeling was developed using a low-temperature diffusive method of radionuclides implementation resulting in $[^{44}Ti]TiO_2$. Chemical composition, particle size distributions and morphology of the radiolabeled NPs remained unaltered compared to the original material. Additionally, $[^{48}V]TiO_2$, which was produced via proton irradiation of TiO_2 NPs (Abbas et al., 2010), was applied within the test systems.
For getting knowledge about transport of TiO_2, interactions of relevant concentrations of these NPs with environmental media (such as humic acids or natural sediments) were studied. Results show that depending on geochemical conditions, transport of TiO_2 in groundwater sediments can be expected, especially in presence of humic acids which act as natural stabilisers for the NPs.
Another important aspect is the ecotoxicological impact of the released NPs. As TiO_2 NP aggregate and sediment from the water column, exposure of benthic organisms to TiO_2 NP is expected. Exposure of $[^{48}V]TiO_2$ NP to the nematode Plectus aquatilis resulted in bioconcentration of the $[^{48}V]TiO_2$ NPs by the nematode, which indicates that transport of TiO_2 NPs up the food chain is possible.
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.

Abbas et al. (2010) J Nanopart Res 12:2435-2443.

N° O3b-1

SONICATION EFFECTS ON MULTI-WALLED CARBON NANOTUBE CHARACTERISTICS FOR TOXICITY STUDIES

S.Allard, S. Che Mansor, M. Ferrie, M. Mayne-L'Hermite, M. Pinault, C. Reynaud, Laboratoire Edifices Nanom

Studies designed to investigate toxicological effects of carbon nanotubes (CNT) rely on the use of ultrasound (sonication) to prepare test suspensions or dried powder batches. However, sonication may alter physicochemical properties of dispersed CNT.
The aim of the present study is to investigate the effects of sonication duration of a CNT suspension in toluene on its length, chemical surface and structure. Toluene liquid medium was chosen as the liquid medium to individualize CNT because of its volatility enabling to remove it easily and therefore to get dried powders. In this context, the approach was to establish a suitable and reproducible protocol for the preparation of dried CNT of a given length which will be further used as dried powders or dispersed in biological medium (water/BSA) for toxicological or bio-distribution tests. .
CNT used in this study has been synthesized by aerosol-assisted catalytic chemical vapor deposition process [1,2]. They are multi-walled carbon nanotubes (MWNT) vertically aligned. Their mean diameter measured by TEM is about 25 nm and their initial length 500 µm. MWNT are dispersed in toluene by using an ultrasonication probe and without any surfactant in order to individualize them and to adjust their mean length to two given values: 1µm and 4µm. In addition, metal pollution (especially by titanium adjunction from the sonication probe) during ultrasonication is measured and a protocol is established in order to remove it. Four suspensions of each length were realized and confirmed the reproducibility of the protocol.
The effect of sonication duration is evaluated on length distribution, structure, purity and surface chemical analysis. CNT length was measured as a function of sonication duration through SEM observations. Metal content (iron residual catalyst and titanium) was measured by TGA under air. Structure and chemical surface were analyzed by Raman spectroscopy and XPS respectively.
The effects of sonication duration which affect both CNT length distribution and crystalline structure as well as chemical surface state will be presented. Microscopic contamination of metal titanium that broke away from the metal probe into the suspension was found. A protocol to obtain dried CNT exhibiting a controlled length distribution and a dispersion protocol to get CNT suspensions in BSA free of titanium pollutant for toxicity studies will be presented.

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N° O3b-2

IGNITION AND EXPLOSION PROPERTIES OF DIFFERENT TYPES OF NANO-MATERIALS

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Nanotechnology is one of the fastest growing industries at present and probably will be for the next decades. This development particularly results from the modified properties of materials within the nanometre range. The different types of behaviour of such substances and the lack of knowledge about their interaction with the environment require intensive investigation for risk assessment.In depth experience and knowledge of fire and explosion risks in the production, handling and transport of nanoscale materials and particles with sizes below one micrometer are not widely available.

This paper describes experiences and results of experiments with several different dusts within the nanometre range. The metallic nano-dusts (aluminium, iron, zinc, titanium and copper) and organic dusts (cellulose, carbon nanotubes) were tested in a modified experimental setup for the test apparatus 20-L-Sphere (also known as 20-L Siwek Chamber), that enables the test samples to be kept under inert atmospheric conditions nearly until ignition. It was designed to allow the determination of safety characteristics of nano-powders under most critical circumstances (e.g. minimisation of the influence of oxidation before the test itself).

Furthermore additional tests with deposited dust and a thermal imaging camera were carried out to describe the burning behaviour of all dusts. For a better characterisation all samples were tested with a Simultaneous Thermal Analysis (STA) and a Bomb Calorimeter.

Experiments conducted so far show that metallic dusts within the nanometre range do not generally possess a higher explosion severity (maximum over pressure and maximum pressure rise) than comparable dusts within the lower micron range. Nevertheless all tested dusts except nano copper are highly ignition sensitive or react pyrophoric. Therefore special measures have to be taken. Consequently under specific circumstances some nano-dusts cannot be tested with current standard test methods for the determination of safety characteristics. As a result with increasing amounts of dust produced within the nanometer range new ways to determine the explosion behaviour of these dusts have to be found. More details of all results will be given in the paper.

Dust sample [a]	Agglomerate Size [µm]	Specific surface area (BET) [m²·g]	p_{max} [bar]	K_{St} [bar·m·s^{-1}]	LEL [g·m^{-3}]
Aluminium (50-70 nm)	4.51	15.20	8.8	421	30
Aluminium (90-110 nm)	4.45	12.25 m²/g	9.1	369	30
Zinc (90-150 nm)	12.68	3.20	4.3	177	125
Copper (50-70 nm)	2.94	7.11	0.3	5	500
Titanium (60-80 nm)	1.24	-	-	-	30
Iron (50-70 nm)	1.27	6.11	-	-	60
Iron (90-110 nm)	1.29	6.42	-	-	60
Multi Walled CNT (ø 50 nm)	23.95	189.34	6.8	68	-
Ultrafine Cellulose (8 µm)	9.43 (no agglomerates)	4.83	9.1	208	-

Results of Testing in 20L Sphere

***Keywords**: dust explosion, nano dust, ignition, explosion protection, cellulose*

N° O3b-3

CHARACTERIZATION OF NANOPARTICLE SIZE AND STATE IN NANOTOXICOLOGICAL AND ECOTOXICOLOGICAL STUDIES USING NANOPARTICLE TRACKING ANALYSIS (NTA)

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Rapid growth in the development and use of engineered nanoparticles continues to run ahead of methodologies for the assessment of the risks they may pose. Awareness grows that the potential long-term toxic effects or environmental impact of such materials are poorly known. Consequently, there is intense research activity about potential toxicological effects of engineered nanomaterials as a new material.
Before starting any nanotoxicological study, it is imperative to know the properties of the nanoparticles used, and in particular their size, size distribution and concentration in an appropriate test media. Indeed, it is well known that particle size determines diffusion rates, penetration of or exclusion by biological barriers and particulate interactions.
This presentation outlines several examples where nanoparticles and their stability in biologically relevant fluids are evaluated via Nanoparticle Tracking Analysis (NTA).
Complementary to classical light scattering techniques, NTA allows nano-objects to be sized on a particle-by-particle basis, enabling high resolution profile. In addition, NTA generates a number-based measure of the concentration (particles/ml), a valuable function in understanding aggregation and other particle behaviour in environmental systems. Finally, with the use of appropriate fluorescent filters, NTA selectively analyses sub-populations of labelled nanoparticles and characterises nano-objects in complex biological media including cell culture media or protein suspensions (Fig 1).
This example (Fig 1a) displays footage of the particles of interest (fluorescently tagged) in a complex background (foetal bovine serum 100%). The particles are moving under Brownian motion but due to the non-specific objects from the serum, it is impossible to isolate their specific signal. Fig 1b shows that using the appropriate laser and filter, it becomes possible to detect the signal from the labelled particles only. This signal doesn't come from the scattering properties (as shown in Fig 1a), but from their fluorescent capabilities. Therefore, once "isolated" from the background, it is possible to track the particles, determine their size, concentration and figure out whether they interact with the media or if their stability is affected (aggregation, etc.).

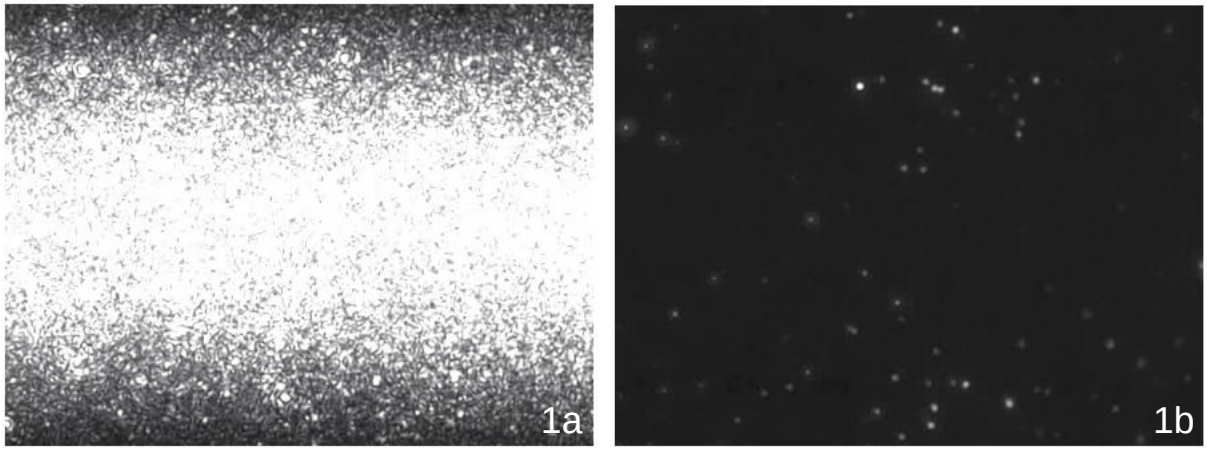

Fig 1 – The use of fluorescence mode allows filtering the signal from a non-specific background.

N° O3b-4

DETAILED CHARACTERIZATION OF WELDING FUME IN PERSONAL EXPOSURE SAMPLES

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Objectives: The objective of the project was to develop a method allowing for detailed characterization of welding particles including particle number concentration, size distribution, surface chemistry and chemical composition of individual particles, as well as metal concentration of various welding fumes in personal exposure samples using regular sampling equipment.

Method: Since the new TLV-TWA for manganese has been lowered to 20 μg/m^3, it is important to have low blank levels for the filters. Uncleaned PTFE and mixed cellulose ester membranes were first tested to determine their blank levels. Filters were digested in a nitric/hydrofluoric/hydrochloric acid mixture and analyzed by ICP/MS. For characterization of particles, welding fumes were collected on polycarbonate membranes using IOM samplers at the Northern Alberta Institute of Technology (NAIT) while students were welding. Five to ten filters were collected, all in the same booth. Simultaneously, number concentration of particles below 1 μm was measured using a P-Trak ultrafine particle counter from TSI. For size distribution analysis, particles were removed from the filters and the solution was analyzed using dynamic light scattering. Filters were inserted into centrifuge tubes with few millilitres of isopropanol, put on a vortex for 2 minutes and sonicated for one hour. Filters were subsequently removed, rinse with isopropanol and discarded.

Preliminary results: Preliminary results for blank filters showed that MCE blanks were significant for all metals analyzed although the blanks were reproducible. PTFE filters showed lower blanks but more variation in the blank. Number concentration of particles was very variable with a maximum around 350,000 particles/cc. The ventilation system was efficient to remove particles during the 15 minutes break students took in the middle of the class, but was not efficient to remove the particles while the students were welding. Removal of particles from filter for size distribution analysis was fairly successful. However, measuring the size distribution of particles using dynamic light scattering was not working well for welding fumes although two different instruments were tested.

Conclusion: For metal analysis MCE filters are not appropriate due to elevated blank levels. Uncleaned and cleaned PTFE and polycarbonate membranes will be analyzed to evaluate their blank levels. Particle concentrations in the welding shop were very high while students were welding even with the ventilation system working. Detailed characterization of particles will be performed using various microscopic techniques: scanning electron microscopy (SEM), X-Ray Photoelectron Spectroscopy (XPS), and X-Ray diffraction XRD. Future work includes the evaluation of using a cascade impactor to determine the size distribution of particles, the reproducibility of the method, and the evaluation of various samplers.

N° O3c-1

HYPHENATION OF NTA ON-LINE WITH AF4/MALS/ICP-MS FOR THE CHARACTERISATION OF NANOMATERIALS IN A COMPLEX MATRIX

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Here we present, for the first time, the potential of hyphenation of nanoparticle tracking analysis (NTA) platform to asymmetric flow field flow fractionation (AF4), multi-angle light scattering (MALS) and as inductively coupled plasma mass spectrometer (ICP-MS) for the characterisation of silica particles, in terms of size, size distribution, size-based elemental composition and number-based concentration in a pure bovine serum matrix. The degree of selectivity offered by AF4 was found necessary to determine accurate number-based concentration of the silica particles in the complex matrix by NTA with an RSD (n=3) ≤ 5% and without any sample pre-processing, i.e. extraction from the matrix, required. ICP-MS detector provided information on size-based elemental composition and element concentration in the separated fraction, as well as particle number. MALS offered complimentary information on particle size, molecular weight and valuable insights into characteristics of the NP capping layer. Comparison of the number-based concentration data obtained by on-line NTA and ICP-MS detectors as well as on the sizing data obtained by NTA and MALS is shown. Such study could only be performed with an on-line approach, as it requires time-resolved influx of sample into all of the detectors within a single measurement.
In summary, NTA hyphenated to AF4/MALS/ICP-MS offers potential for reliable NP size and number determination in complex samples and could support current and upcoming regulations concerning commercial use of nanomaterials.

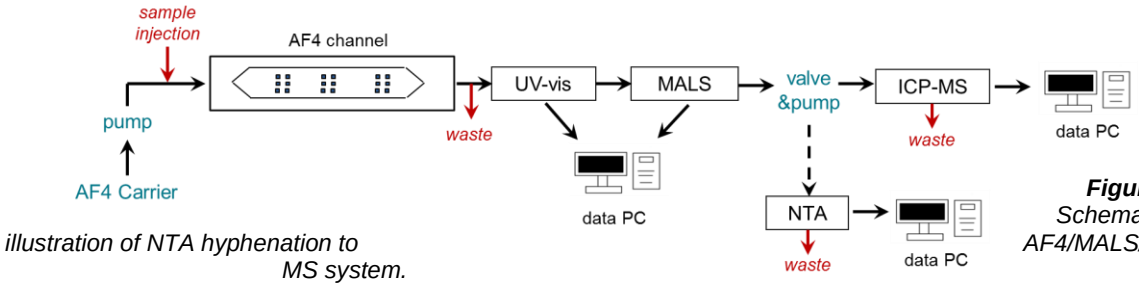

***Figure 1**. Schematic illustration of NTA hyphenation to AF4/MALS/ICP-MS system.*

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N° O3c-2

TOWARDS ROUTINE NANOPARTICLE MEASUREMENTS WITH PERSON-CARRIED INSTRUMENTS

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The personal measurement of nanoparticles or ultrafine particles can still not be regarded as routine. Several research projects have done their share to improve the situation. To name but two: Nanodevice within the 7th European Research Framework and NanoGEM a German project have successfully managed to improve availability, quality control, and ease of use of person carried instruments for the task.
Currently these projects have found successors to maintain momentum towards a routine application of nanoparticle personal measurements. We report here about NanoIndEx and two work packages within NanoCEN. NanoIndEx is a consortium formed as an ERA SIINN network with the aim of developing and describing routine standard operation procedures for personal measurements of nanoparticles, whereas NanoCEN is part of the very large European mandated standardization effort M/461 "Standardization activities regarding nanotechnologies and nanomaterials". The latter contains among other workplace-exposure related topics two subprojects which deal with the standardization of the use of condensation particle counters in workplace measurements (“Characterization of ultrafine aerosols/nanoaerosols – Determination of number concentration using condensation particle counters" and surface measuring devices.”) and the development of recommendations for the particular metric to be used in exposure measurements (“Guidance on metrics to be used for the measurements of exposure to inhaled nanoparticles (nano-objects and nanostructured materials) such as mass concentration, number concentration and surface area concentration”).
Work in these three projects has started. All of them make use of a tool developed in Nanodevice and NanoGEM, the Nano Test Center of IGF, extensively described elsewhere [Lit]. Within 3 large round robin tests a number of various CPCs and diffusion charging instruments have been tested to develop standard operation procedures for their use, which should ultimately lead to their publication as CEN standards within working group 3 of TC 137 “Assessment of workplace exposure to chemical and biological agents – Particulate Matter”. Aim of these round robin tests is to find well practicable procedures for personal measurement of nanoparticles (NanoIndEx), conditions of use for condensation particle counters and diffusion charging instruments (NanoCEN) under which they may be used reliably for the purpose.
The results of these round robin tests and first recommendations are reported.
Acknowledgements:
The research leading to these results has received partial funding from the European Mandate M/461 "Standardization activities regarding nanotechnologies and nanomaterials" contract M/461, SA/CEN/ENTR/461/2012-06 (NanoCEN). NanoIndEx is supported by the French National Funding Agency for Research (ANR), the German Federal Ministry of Education and Research (BMBF), the British Technology Strategy Board (TSB) and the Swiss TEMAS AG, under the frame of SIINN, the ERA-NET for a Safe Implementation of Innovative Nanoscience and Nanotechnology.
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N° O3c-3

DESIGN OF AN EXPOSURE CHAMBER FOR EVALUATION OF PERSONAL SAMPLERS

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According to the National Institute for Occupational Safety and Health (NIOSH), there are more than 400,000 workers worldwide that are exposed to nanoparticles. However, there is no standardized method to assess workers' exposure to nanoparticles. It is therefore paramount to develop a proper sampling and analytical method. Personal samplers are usually utilized for collection of different micro and nano particles present in a working environment. Collected particles can then be characterized by different techniques. Particles properties such as size, size distribution, aspect ratio and surface properties are important in terms of the efficiency of a sampler. In order to reduce measurement errors and obtain most accurate results one should have an understanding of the efficiency of the sampler. This is normally done by exposing particles with known properties to a sampler and then evaluating the sampler efficiency by analysing the collected particles. Therefore to evaluate the performance of a sampler there is a need for a chamber that is capable of providing a controlled environment with a uniform distribution of particles with known properties. In this regard the aim of this study was to design a laboratory size exposure chamber for testing of personal samplers.
A polyethylene cylindrical container with a diameter of 42 cm and height of 60 cm was used as the testing chamber. The chamber was divided into 2 parts by an aluminium honey comb. Particles generated using a 1 jet Collison nebulizer (BGI) operating at a flow rate of 4L/min were inserted into the chamber via a tube located near to the top of the chamber. As particle charge can dramatically affect sampling a particle neutralizer was attached to the generation system so as to neutralize the particles before they enter the chamber. One air inlet was also placed at the same height for dilution. Diffusion dryers were used to remove any water from the air streams prior to enter the chamber. A fan was used to mix and distribute the generated particles. After generation and mixing, the particles passed through the aluminium honeycomb which is essential to eliminate any turbulent or unwanted air flow. Six sampling ports along with a pressure gauge were placed on the walls 15 cm from the bottom of the chamber. The pressure gauge was added to ensure the desired pressure is achieved during sampling. The sampling ports allowed for the connection of five samplers and sampling pumps as well as the connection of an ultrafine particle counter (P-Trak, TSI). The exposure chamber was developed to assess various samplers for carbon nanotubes and cellulose nanocrystals.

N° O3c-4

DESIGN OF NANOPARTICLE REFERENCE MATERIALS FOR SENSOR DEVELOPMENT IN THE CONTEXT OF THE EU-PROJECT INSTANT

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Despite ongoing efforts the detection of engineered nanoparticles (ENPs) in complex media as cosmetics, foods or beverages is still remaining an important analytical challenge. ENPs may occur in such matrices unintentionally due to production processes, migration from packaging or may be added e.g. to adjust thixotropy, as anti-caking or colouring agents.
The EU-project INSTANT (http://www.instant-project.eu) which is an acronym for *Innovative Sensor for the fast Analysis of Nanoparticles in Selected Target Products* is dealing with the development of an integrated system to extract, enrich and detect arbitrary nanoparticles in such complex matrices.
The sensor array of the INSTANT device is based on molecular imprinted polymers (MIPs) with opto-electrochemical readout.
Therefore, standard nanoparticles of different physicochemical identity are synthesized and characterized at BAM, tailored to different sizes, small size-distributions and certain shape while bearing different stabilizer materials and exhibit sufficient stability.
First, those particles are needed for the system development in terms of MIP production as well as calibration and testing of the INSTANT device on the other hand, new nano reference materials which are available beyond the duration of the project in sufficient quantity, are produced and tested for quality and long-term stability by means of small-angle X-ray scattering (SAXS) and dynamic light scattering experiments (DLS).
The reference material development in the scope of the INSTANT project at BAM is depicted and a first candidate reference material of poly-N-vinyl pyrrolidone (PVP)-decorated spherical silver nanoparticles, currently under development, is presented.

N° O3c-5

MULTI-INSTRUMENT MANAGER TOOL FOR DATA ACQUISITION AND DATA MERGING OF OPTICAL AND ELECTRICAL MOBILITY SIZE DISTRIBUTIONS

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The measurement of number concentrations and size distributions of aerosols in the sub-micrometer range has received much attention during the last years. Assessing possible exposure to nanomaterial used in or added to products and the investigation of airborne nanoparticles requires suitable, sensitive instruments. Commonly electrical mobility classification followed by Condensation Particle Counter (CPC) detection is the technique of choice. Both are typically combined in a Scanning Mobility Particle Sizer (SMPS) to retrieve nanoparticle size distributions in the range from 2.5 nm to 1 µm. During the last NanoSafe Conference we introduced the NanoScan SMPS (TSI model 3910, Tritscher et al., 2012), a novel, portable nanoparticle sizing and counting instrument in the range from 10 to 420 nm. Its portability is a key to help in source identification.

The detection size range of SMPS systems can be extended by the addition of an Optical Particle Sizer (OPS, TSI model 3330) that covers larger sizes from 300 nm to 10 µm and is also portable and compact designed. This optical sizing method reports an optical equivalent diameter, which is often different from the electrical mobility diameter measured by the standard SMPS technique.

Multi-Instrument Manager (MIM) software was introduced by TSI development of algorithms that facilitate merging SMPS data based on electrical mobility diameter with data based on optical equivalent diameter to compile a single, wide-range data set. Here we present MIM 2.0, the next-generation of the data merging tool that offers many advanced features for data merging. MIM 2.0 allows direct data acquisition with OPS and NanoScan SMPS instruments to retrieve real-time particle size distributions from 10 nm to 10 µm.

The MIM software tool is MATLAB-based and also allows reviewing and averaging data from TSI SMPS spectrometers, the NanoScan SMPS and/or OPS. MIM 2.0 features increased channel resolution of up to 128 size channels per decade of recorded SMPS raw data files, comparison of SMPS correction steps and combination of multiple units. One of the key features when merging OPS with Electrical Mobility distributions is that the software takes aerosol optical properties into account by automatically determining an overall aerosol effective refractive index that was used to adjust the merged data. Thus an indirect and average characterization of aerosol optical and shape properties is possible. In Figure 1 we show an example for measurements from NanoScan SMPS and OPS with the resulting composite fit. The merging procedure is heavily dependent on the particle type, concentration and distribution. MIM 2.0 allows several pre-settings, data averaging and adjustments, as well as data set and fitted graph export.

Our presentation will show SMPS post-processed data as well as different merged data sets and merging options to demonstrate their impact on the final particle number size distribution.

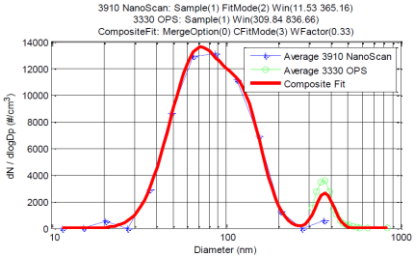

Figure 1: Merged data example (red) with the original (averaged) particle number size distribution from the NanoScan SMPS shown in blue and from the OPS in green.

N° PL4

NANOPARTICLE TOXICOLOGY: A CRITICAL APPRAISAL OF HAZARD AND RISK CHARACTERIZATION

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Nanotoxicology is maturing into a widely applied discipline by designing appropriate *in vitro* and *in vivo* testing strategies to identify and characterize potential hazards due to exposure of humans and the environment to nanomaterials and to uncover mechanisms that may trigger adverse effects. However, despite - or because of - this growth there are frequently misunderstandings regarding concepts and the proper use of nanotoxicological methods, devising appropriate studies, analyzing and interpreting results or characterizing hazard and risk. Some concepts are uncritically accepted as dogma rather than being rejected because of misunderstanding or uncritical assumptions or ignorance. As a result, certain misconceptions are perpetuated in many peer-reviewed publications as established facts and are presented without a necessary critical discussion. Issues that are addressed include dosing and dosimetry of the respiratory tract including overload and the role of physico-chemical properties as dosemetric; *in vitro-in vivo* dosemetric correlations; dosimetric extrapolation and comparative hazard and risk characterization; hazard identification and ranking based on the concept of benchmarking. Concepts need to be scientifically validated, supporting and opposing arguments should be carefully evaluated. A critical appraisal of all phases of study designs and data interpretation is necessary when characterizing hazard and risk of nanomaterials due to environmental or occupational exposures.

N° O4a-1

WHAT IS THE IMPACT OF CARBIDE NANOMATERIALS TO THE MINERAL COMPOSITION OF RAT LUNGS? A PIXE-µPIXE COMPARATIVE STUDY

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Several engineered nanomaterials (ENMs) are of an insoluble to traditional chemical digestion techniques rendering traditional chemical analytical methods unsuitable for *ex-vivo* organ biopersistence studies. Ion beam techniques like Particle-Induced X-ray Emission (PIXE) and micro-PIXE (µPIXE) can provide together global concentrations and local distribution maps of NMs. The advantages of PIXE for this kind of measurements are its non-destructive nature, multi-element acquisition, ppm-levels of sensitivity and the capacity to measure ENMs of any size due to the nature of ion-matter interactions. µPIXE has the same advantages o PIXE plus the capacity to generate 2D elemental maps with micron-lateral resolution (Grime et al, 2014). It is of interest, on top of having ENMs concentration maps and global values, an analysis of the effects of the exposed tissues such as changes in their elemental concentration (Lozano et al, 2012).
In this study Sprague-Dawley rats were exposed to a nanoaerosol of SiC ENMs in an acute exposure and sacrificed at selected days afterwards. Rat lungs were prepared for µPIXE or PIXE and chemical elemental analysis was carried out by the GUPIXWIN software. Statistical analysis was applied to study the elemental differences between exposed and control lungs to elucidate the effects on the lungs chemical composition. The results will be presented in light of other recent studies discussing the change in organ elemental composition.

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N° O4a-2

THE IMPACT OF SIC AND TIC NANOMATERIALS IN A RAT MODEL

Omar Lozano[1], Julie Laloy[2], O. Toussaint[3], Jean-Michel Dogné[2], Stéphane Lucas[1], (1) Research Centre for the Physics of Matter and Radiation (PMR), (2) Department of Pharmacy, Namur Medicine Drug Innovation Center (NAMEDIC), (3) Laboratory of Biochemistry and Cellular Biology (URBC), all of Namur Nanosafety Centre (NNC), Namur Research Institute for Life Sciences (NARILIS), University of Namur (FUNDP), Rue de Bruxelles 61, B-5000 Namur, Belgium.

Silicon carbide (SiC) and Titanium Carbide (TiC) are widely used on industrial scale, for example in mining and petroleum sectors. When these products wear, have a breakdown or undergo decommissioning there is a risk that particle detachment takes place with a size distribution including the nanoscale. Given the higher associated risk with nanomaterials (NMs) and the high use of SiC and TiC industrial products, toxicological and physicochemical studies exploring the pulmonary and oral exposures routes in a rat model were performed in order to assess the toxicokinetics and toxicodynamics under such exposure conditions.
Female Sprague-Dawley rats were exposed to either SiC or TiC in form of nanoaerosol or by oral gavage under acute and subactute conditions and sacrificed immediately after exposure or at selected days later. Rat tissues and fluids were submitted to extensive physicochemical and classical toxicology studies: NM biodistribution by Particle-Induced X-ray Emission (PIXE) and electron microscopy (EM), histophatological examination, plasma and urine analysis, and broncheoalveolar lavage.
The main results indicate lung inflammation, the capacity of trespassing the gastrointestinal tract and alterations in the mineral uptake and organ content within the rat model. These results will be presented in detail and compared in light to other more common NMs such as SiO_2 and TiO_2.

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N° O4a-3

ORGAN WEIGHT CHANGES IN MICE AFTER LONG-TERM INHALATION EXPOSURE TO MANGANESE OXIDES NANOPARTICLES

Tomáš Zeman[3], Marcela Buchtová[1], Bohumil Dočekal[2], , Ivan Míšek[1], Pavel Mikuška[2], Omar Šerý[1], Zbyněk Večeřa[2], (1) Institute of Animal Physiology and Genetics, v.v.i., Academy of Sciences of the Czech Republic, Veveří 97, 602 00 Brno, Czech Republic (2) Institute of Analytical Chemistry, v.v.i., Academy of Sciences of the Czech Republic, Veveří 97, 602 00 Brno, Czech Republic (3) Department of Population Protection, University of Defence, Kounicova 65, 662 10 Brno, Czech Republic.

The aim of the study was to assess effect of inhaled manganese oxides nanoparticles on weight of internal organs. For this purpose a long-term inhalation experiment on laboratory mice was performed under controlled illumination, temperature, relative humidity and pressure.
Manganese oxides ($MnO.Mn_2O_3$) nanoparticles (MnONPs) were synthesized continuously via aerosol route in a hot wall tube flow reactor using thermal decomposition of metal organic precursor manganese(II)acetylacetonate in the flow tube reactor (in vertical position) at temperature 750 °C in the presence of 30 vol% of oxygen. The concentration of produced MnONPs at the reactor output was in the range $1–3 \times 10^7$ particles/cm^3 and the size of generated nanoparticles $MnO.Mn_2O_3$ was in the range 7–50 nm. Before entering the inhalation chamber, MnONPs in the mixture of N_2, O_2 and air (at total flow rate of 3 L/min) were further diluted using filtrated humidified air (at flow rate up to 60 L/min) at temperature 21 °C resulting in the MnONPs concentration $5 \times 10^5–2 \times 10^6$ particles/cm^3. The experimental group of mice was exposed to MnONPs for 17 weeks. At regular time intervals some of the mice were removed, autopsied and their internal organs were weighed.
It has been proven that inhaled nanoparticles are able to influence the weight of internal organs of mice. The mice from the experimental group had statistically significantly lighter kidneys, liver and spleen and heavier pancreas compared to the mice from the control group.
This study was supported by the Grant Agency of the Czech Republic (P503/12/G147, P503/11/2315) and the Ministry of Education, Youth and Sports of the Czech Republic (SV14-FEM-K106-08-ZEM).

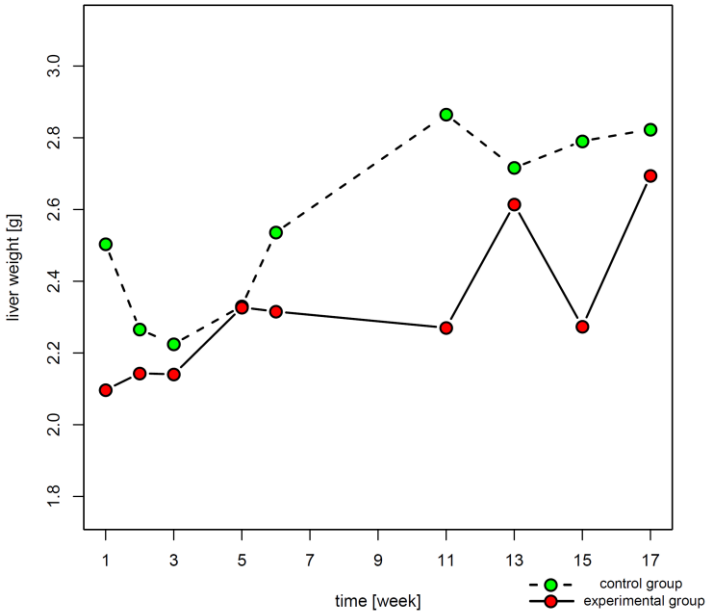

Fig. 1 Median liver weight of mice in the course of the experiment; green points represent the medians of the control group; red points represent the medians of the experimental group; solid lines connect the medians of the control group; dashed lines connect the medians of the experimental group.

N° O4a-4

SILICA NANOPARTICLES CAUSE PLEURAL EFFUSION, PERICARDIAL EFFUSION AND PULMONARY FIBROSIS IN RATS

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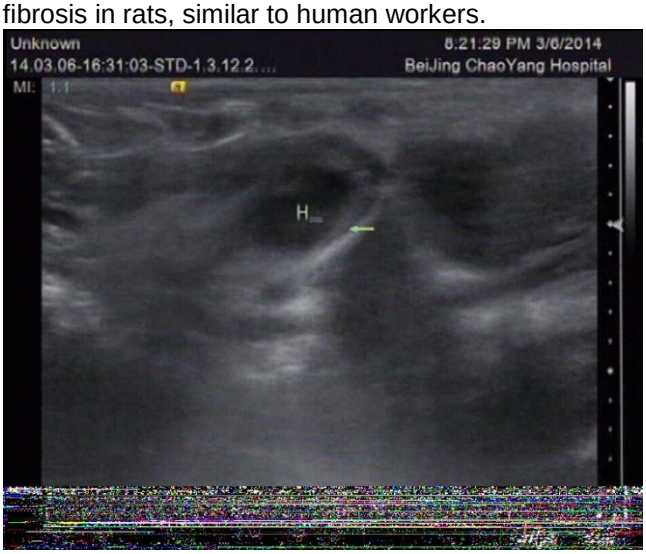
Nano materials generate great benefit as well as potential damage to humans. We have ever reported a group of workers exposed to nano materials with unusual disease - pleural effusion, pericardial effusion and pulmonary fibrosis, and silica nanoparticles were isolated and identified in patients' lungs and pleural effusions. The study aims to testify if silica nanoparticles can cause similar symptoms of pleural effusion or pericardial effusion in rats.
72 male Wistar rats were divided six groups, each with 12 rats: blank control group, control group with normal saline, control group with polyacrylate solution, and silica nanoparticle group of low, intermediate, and high-dose. Silica nanoparticles (20nm) were intratracheally instilled into rats at doses of 3, 6, or 12 mg/kg. Pleural effusion and pericardial effusion were detected by the ultrasonic examination on day 3,5,7,14,21, 28 of post-instillation. And CT scans were conducted to probe into the damage of lungs or pleural effusion on day 14 and day 28 after instillation. Three rats of each group were executed on day 3,7,14, or 28, and light microscopic study and transmission electron microscopic examination were performed.
Pleural effusions were observed in the groups of silica nanoparticles on day 5 after instillation and lasted for 14 days, pericardial effusions in the intermediate and high dose group of nanosilica, but not in the low dose group, were also observed, neither pleural effusions nor pericardial effusions were observed in the control groups. And rats' pulmonary and pleural fibrosis was also found in silica nanoparticle exposure group. We conclude that silica nanoparticles cause pleural effusion, pericardial effusion and pulmonary fibrosis in rats, similar to human workers.



A great amount of pericardial effusion was observed in a rat on day 5 after instillation of silica nanoparticles.

N° O4b-1

INTERLAB STUDY ON NANOTOXICOLOGY OF REPRESENTATIVE GRAPHENE OXIDE

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Graphene oxide (GO) is a material with potential in many different applications and with thermal stability (Zhou et al. Sci. Rep. 3:2484 (2013)). GO is not considered till now as representative compound in the actual studies in the EU (e.g. REACH, OECD). Recently, European Commission's JRC Institutes IRMM (Belgium) and IHCP (Ispra, Italy) introduced the term 'representative test material' (RTM), and provided a frame for its use (Roebben et al. J. Nanopart. Res. 15, 1455 (2013)). The definition of RTM is a material from a single batch, homogeneous and stable with respect to one or more specified properties (ISO/AWI TS 16195). The ISO Tech. Comm. Nanotechnol. (TC 229) has recently published a new standard: ISO/TR 16197:2014 – Nanotechnologies – Compilation and description of toxicological screening methods for manufactured nanomaterials. This standard is a Technical Report related to *in vitro* and *in vivo* methods that can be useful for the toxicological and ecotoxicological measurement, complementing the ISO/TS 27687:2008 and ISO/TS 80004-1. In view of these documents, our Brazilian Network on Nanotoxicology-GIGENANOTOX (MCTI/CNPq) decided to follow and evaluate these new rules for a selected representative compound, GO, by different nanotoxicological assays. The graphene sample GO:Single-layer graphene oxide, purity 99%, thickness 0.7-1.2 nm (AFM); ~300-800nm X&Y dimensions is the standard size <450 nm & 1-20 μm lateral dimensions. Cheap Tubes Inc., Bratleboro, USA was selected for our study. Exhaustive characterization of GO was afforded. It exhibited thermal stability over 60^{o}C and it was suspended in deionized water after ultrasonication (1 mg/mL) (stable 10 days). All the biological fluids used in the different assays were used as control of the colloidal suspension stability. Then, all the studies were carried out within that stability period. The cytotoxicity assays were carried out by the Rezasurin reduction, MTT and flow cytometry assays in mouse embryonic fibroblast cells (3T3), human keratinocytes (HaCaT), colorectal cancer cells (Caco-2/HCT 116), Lewis lung cancer cells (3LL), acute *myeloid leukemia* cells (KG-1, Jurkat, Kasumi-1) and chronic myeloid leukemia cells (K562, Lucena) and no significant toxicity was found after exposition to 0.1-100 μg/mL for 24 and 48 h. Breast cancer cells, MCF-7, showed a 20% reduction on cell viability at 24 and 48 h. No cytotoxicity were found in lymphocytes, Chinese hamster ovary cells (CHO) and human macrophage cell line (U937) at 0.1-50 μg/mL, but 30-50% survival inhibition was observed at 100 μg/mL. A dose-dependent increase in apoptosis was observed in some cells (Kasumi-1, Jurkat and K562 cells). In the case of CHO and 3T3 cells, greater levels of necrosis with increasing concentrations of GO (>50 μg/mL) were observed. Genotoxic study using the Comet assay showed slight DNA damage in lymphocytes at all concentrations tested, while more significant effects were observed in 3T3 cells, being worst in CHO cells. Econanotoxicity was carried out by lethality assays in the nematode *Caenorhabditis elegans* and in the freshwater coelenterate *Hydra* with no signs of toxicity at concentrations varying from 0.1-50 μg/mL of GO. However, death and disintegration of *Hydra* was observed after exposition to 100 μg/mL for 72 h. In *in vivo* studies, no changes in biochemical parameters of Fischer 344 rats were observed after the i.p. administration of GO, suggesting that it is less efficiently removed by the reticuloendothelial system (RES), probably due to agglomeration after the i.p. injection, thus leading to low absorption. Black agglomerates were indeed found in the intraperitoneal cavity of rats injected with GO. This was also observed previously in Balb/c mice receiving GO i.p. (Yang et al., Biomaterials 34, 2787 (2013)). However, in Fisher 344 rats-bearing prostate tumors, treatment with GO negatively affected the hepatic parameters, whilst in the renal ones, an improvement was observed. Studies are in progress to understand the mechanisms involved in the uptake of GO by RES. The validation of all of these assays involving GO will be discussed in details in the presentation.

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N° O4b-2

BIOLOGICAL RESPONSE TO PURIFICATION AND ACID FUNCTIONALIZATION OF CARBON NANOTUBES

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1.Figarol, A. *et al.* Biological response to purification and acid functionalization of carbon nanotubes. *J. Nanoparticle Res.* **16,** 1–12 (2014).

Due to their exceptional physiochemical properties, carbon nanotubes (CNT) raise great industrial and research interests in fields as diverse as biomedicine, microelectronics or composites. Surface functionalization by acid treatment has been described as an easy way to reduce the CNT hydrophobicity and biopersistance. Its impact on *in vitro* cellular responses remained however unclear with numerous contradictions between studies.

This work aimed to provide a comprehensive analysis of the acid functionalization effects on cellular activity. The impact of (1) the CNT purification from their catalytic impurities and (2) the presence of surface acid groups have been discriminated. A thermal desorption treatment to remove surface acid groups has been conducted on part of the CNT after acid functionalization by HNO_3, H_2SO_4. It left all other physicochemical characteristics unchanged, and allowed a monofactorial analysis of the effects of the surface acid groups. After a complete physicochemical characterization, the biological toxicity was evaluated on RAW 264.7 murine macrophages, comparing (1) the pristine and desorbed CNT, and (2) the functionalized and desorbed CNT.

Results showed that acid functionalization leads to (1) a decrease of the pro-inflammatory response, oxidative stress and to a lesser extent cytotoxicity due to the CNT purification, and (2) an increase of the pro-inflammatory response due to the surface acid groups. This study, published in *Journal of Nanoparticle Research* [1], underlined the necessity to weight the benefit-risk balance of such an acid purification based on the CNT primary state of purification for a safer-design approach.

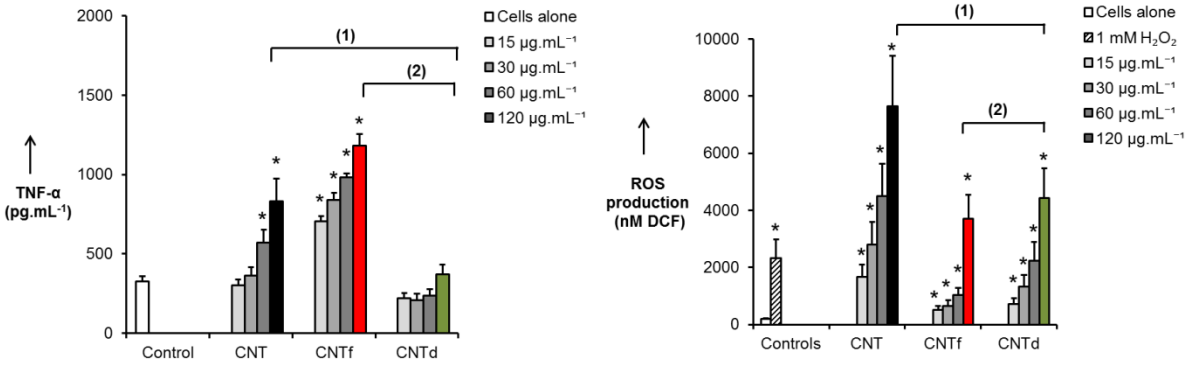

Figure 1: *Pro-inflammatory and oxidative stress responses to pristine carbon nanotubes (CNT), acid functionalized carbon nanotubes (CNTf) and functionalized then thermally desorbed carbon nanotubes (CNTd).*

N° O4b-3

CYTOTOXICITY EVALUATIONS OF CARBON DOTS WITH DIFFERENT SURFACE CHARGE

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Carbon dots are a new class of the fluorescent nanoprobes which have been found promising for bio-applications. Surface functionalization plays a critical role in the cytotoxicity determination of carbon dots and is a very important issue. One of the main factors which affect the cytotoxicity is a surface charge as influences cellular/intracellular tracking of nanomaterials. Thus, a comparative study of C-dots with different surface charges was performed on standard cells mouse fibroblasts (NIH-3T3). We tested pristine carbon dots derived from candle soot with the same C-dots functionalized with polymers. Polyethilenimine (PEI) and polyethylenglycol (PEG) have been used to obtain different surface charges of C-dots (PEI – positive charge, PEG neutral charge); pristine C-dots bear negative charge by themselves. Changes of viability were measured by MTT assay, oxidative stress was determined through ROS analysis, and the cell cycle profile was obtained by flow cytometry analysis. Morphology changes in the cells caused by incorporated carbon dots were observed by light microscopy. From cytotoxicity measurement, we identified that the surface chemistry affects viability and oxidative stress. The most toxic response was induced by positively charged PEI-carbon dots at a concentration of 50 ug/ml. Viability of the cells loaded by PEGylated carbon dots did not decrease significantly up to 300 ug/ml. The bare C-dots stimulated proliferation at the low concentration until 100 ug/ml and then viability went down with increasing concentration (IC_{50} value was found at 300 ug/ml). ROS kinetic study has shown that the highest oxidative stress occurred in cells labeled with pristine carbon dots. PEGylated C-dots did not cause a considerable ROS level compared to the control cells. From the cell cycle analysis, we evaluated the arrests in the individual phases. PEGylated carbon dots did not display any abnormality in proportion of the cell cycle. Cells treated with 50 ug/ml of the PEI C-dots tend to be arrested in G0/G1 and the G2/M phase became relatively more populated than the S phase. Pristine carbon dots did not affect the cell cycle significantly at a concentration of 50 ug/ml but at 100 ug/ml, the G2/M arrest occurred. The arrest in G0/G1 occurred at the 350 ug/ml treatment. We carried out the comprehensive cytotoxicity study and found out a different biocompatibility of carbon dots depending on the surface chemistry.

N° O4b-4

INTERACTION OF NANOMATERIALS WITH TOLL LIKE RECEPTOR LIGANDS: CHARACTERIZATION OF THE BIOMOLECULE CORONA AND INFLUENCE ON THE CELLULAR RESPONSE

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At the nanoscale, materials exhibit unique physicochemical properties, and as their surface to mass ratio is much higher compared to larger materials the adsorption of biomolecules onto a nanomaterial (NM) surface is considered to be greater. Through hydrogen bonds, van der Waals interactions and solvation forces NMs can easily interact with naturally occurring biomolecules during the NM production, application and disposal, which may have a negative impact on the health of industry workers, consumers and the environment. So far, even for nanosafety studies, NMs are only routinely tested for contamination with lipopolysaccharides (LPS), a component of the outer membrane of Gram-negative bacteria, while there are many other biomolecules present which also impact upon the immune system and thus far go usually untested.
The aim of this study was to determine the necessity of testing additional bacterial substances, which are able to interact with the NMs during the different stages of their life cycle and additionally, to determine if the conjugation of those substances to the NM alters the cellular response.
The biomolecules used in this study were LPS, CpG-DNA, flagellin and ssRNA. All these substances are bacterial/viral-derived pathogen-associated molecular patterns (PAMPs) recognized by Toll-like receptors (TLRs) located in the cell membrane or in the endosomal membrane. The substances were incubated under gentle agitation at 4°C overnight with 50 nm gold nanoparticles (AuNPs). After incubation, unbound and loosely bound molecules were removed by centrifugation. Characterization of the PAMPs-AuNP conjugates was performed by dynamic light scattering (DLS) and transmission electron microscopy (TEM) to monitor size increases and to determine dispersion. The amount of bound PAMPs was analyzed by high pressure liquid chromatography (HPLC), and, where appropriate, by limulus amebocyte lysate assay (LAL) and SDS-PAGE. The cellular response was tested via the assessment of NF-κB activation in an overexpression system using transiently transfected human embryonic kidney cells (HEK293).
Negative staining of PAMPs-AuNP conjugates confirmed the presence of a hard corona. A size increase due to the formation of a hard corona was additionally verified by DLS. The exposure of flagellin and CpG-DNA AuNP conjugates led to a decrease in NF-κB activation when compared to the PAMPs alone, whereas an increase in NF-κB activation was shown in response to LPS-AuNP conjugates, which may be due to the high localized concentration. The decrease of response to other factors may be explained by reduced ability of TLRs to recognize the PAMPs when bound to AuNPs, thus leading to an anti-inflammatory response of NPs.
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N° O4c-1

CHARACTERIZATION OF NANOPARTICULATE SURFACES AND THEIR RELATION TO DIFFERENT PROTEIN CORONAE

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Investigation of the toxicity of nanoparticles (NP) is a highly challenging task due to the fact that it does not only depend on the material under consideration but is strongly affected by size, shape, crystal structure and surface modification.[1] In brief, dispersity is a big issue. Regarding the usually applied experimental strategies, high-throughput screenings are often applied to investigate large parameter spaces in terms of their global toxicity.[2] However, those screening approaches need to be supplemented by detailed studies where a mechanistic understanding *e.g.* of the cellular uptake of the material in question is derived. For this step especially the protein corona was shown to be of major importance.[3-4] Thus, the composition of proteins associated to the particle surface will finally decide if NP are transported inside a cell or not. When the NP are inside the cell, a cascade of different oxidative stress levels is passed which in the worst case ends with cell apoptosis.
For the present contribution we focus on the very first step and investigate the relationship between dispersity, the obtained protein coronae in different culture media and its influence on the NP to cell transport. For all our investigations we chose different technically relevant, liquid borne NP materials: ZnS and ZnO semiconductor NP (also known as quantum dots, QDs) exceeding from 1.5 to 7 nm and Au NP exceeding from 5 to 30 nm. They can all be easily detected by UV/Vis spectroscopy due to their pronounced absorption features. Characterization of the QD core particle size distribution (PSD) is easily realized by deconvolution of absorbance measurements[5-6] whereas for Au NP standard techniques like dynamic light scattering (DLS) and electron microscopy are applied.
However, especially for smallest, liquid borne nanoparticles < 20 nm the surface chemistry needs to be considered carefully. NP surfaces strongly depend on the attached ligands which are either present as residuals from the synthesis process or applied in a subsequent decoration step. The latter ensures colloidal stability and adds new functionalities to the system. Unambiguous characterization and control of real particle surfaces at ambient conditions can only be realized by the unique combination of different characterization techniques: in addition to standard ζ-potential analysis, analytical ultracentrifugation (AUC), a combined UV/Vis-ICP approach as well as inelastic Raman scattering give access to the adsorption isotherm. By isothermal titration calorimetry (ITC) complementary heats of adsorption are measured and translated into enthalpy and entropy. In the last step, characteristic fingerprints of protein coronae were derived by gel electrophoresis of NP isolated after their exposure to simulated lung fluid (SLF). SLF represents one of the most important ways of NP infiltration into the human body and is thus of highly relevant.
Our work allows the connection of protein coronae not only to different core materials and core size distributions but also to surface charge and surface chemistry. This is seen as a key step in understanding the formation of protein coronae and makes an important contribution in understanding the cellular uptake of NP.

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N° O4c-2

COMPUTATIONAL METHODS FOR THE TOXICOLOGICAL ASSESSMENT OF MANUFACTURED NANOMATERIALS

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To address environmental and health impacts posed by chemical substances, Human and Environmental Risk Assessment (HERA) is considered a pertinent approach that can be adapted to assess potential risks caused by manufactured nanomaterials (NMs). HERA is a process by which scientific and regulatory principles are applied in a systematic approach in order to address qualitatively and/or quantitatively the hazard that substances may pose to human health or environmental species.
NM safety in the EU is addressed by regulatory frameworks either directly or implicitly. NMs are covered by the substance definition in the Registration, Authorisation and Restriction of Chemicals regulation (REACH, EC No 1907/2006) and are directly addressed by the Cosmetic Products regulation (EC 76/768/EEC, No 1223/2009) and Biocidal Products regulation (EC No 528/2012). These regulatory frameworks are intended to ensure chemical safety, while enhancing competitiveness and innovation. At the same time, they aim to reduce the use of *in vivo* testing and promote the use of alternatives such as *in silico* and *in vitro* methods. This is especially relevant for cosmetics, since a complete ban on animal testing for ingredients and products entered into force in 2013.
The development of valid *in vitro* and *in silico* methods that are applicable to NMs represents a great challenge due to the unique characteristics of these novel materials. Little is known about their fate and behaviour under physiological conditions and their mechanisms of toxicological action (e.g. oxidative stress, irritation and inflammation). Accordingly, few predictive models are available to date.
The NanoComput project, being carried out by the European Commission Joint Research Centre, is aimed at identifying how existing knowledge, data and models may be used to evaluate NM safety and foster safe product design. It will support the development of guidelines for the grouping of NMs in existing regulatory frameworks. It will also investigate the possible application of alternative (non-animal) approaches such as Adverse Outcome Pathways (AOPs), read-across, grouping, quantitative structure-activity relationships (QSARs), possibly with the help of physiologically based toxicokinetic (PBTK) models, and within integrated approaches to testing and assessment (IATA) for NMs.
Read across and QSAR methods will be considered in combination with PBTK approaches to identify possible descriptors that are useful in predicting the toxicological actions of NMs. This will be done taking into account *in vitro* toxicity methodologies in an AOP context, providing a structured overview of relevant physiological events that are related to key adverse outcomes. This effort will support the development of guidance and software tools.

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N° O4c-3

ASSESSMENT OF THE OXIDATIVE POTENTIAL OF NANOPARTICLES: COMPARISON AND IMPROVEMENT OF METHODS

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*Background***:** Oxidative stress induced by reactive oxygen species (ROS) is increasingly being demonstrated as playing a key role in the biological response induced by nanoparticles (NPs). Yet, there is a need to adapt or improve current methods to characterize the intrinsic and cellular oxidative potential of NPs and to develop high-throughput assays to allow the rapid screening to classify NPs in terms of toxicity. For instance TiO_2 and CeO_2 are widely used for industrial, environmental and biomedical applications leading to possible exposures. Therefore, there is a need to assess potential pulmonary responses to NPs and the underlying mechanisms.
Methods: The physico-chemical properties of suspensions of highly oxidative control (Mn_2O_3 NPs), low oxidative widely used control (CeO_2 and TiO_2 NPs) and negative control ($BaSO_4$ NPs) were characterized. Three acellular assays were used in order to measure the intrinsic oxidative ability of NPs: cytochrome c assay was adapted for nanotoxicology purpose and compared to the DTT assay performed in dose-course studies and plasmid DNA assay to investigate oxidative damage to supercoiled DNA induced by free radicals. A high-throughput assay of the cytochrom *c* test was developed in view of its application for nanotoxicology screening strategies. To assess whether the oxidative potential of the tested NPs correlates with cytotoxicity and cellular oxidative stress responses we studied the effect of negative control, high and low oxidative NPs on the human bronchial epithelial cells NCI-H292. The cytotoxicity of the NPs was evaluated by the WST-1 assay. The ability of NPs to induce an oxidative stress *in vitro* was assessed by measuring the induction of antioxidant enzyme expression in NCI-H292 cells treated for different times with NPs and the pro-inflammatory response triggered by NPs was assessed by measuring the Il-8 release by ELISA and mRNA expression by RT-qPCR.
Results: The adapted cytochrome *c* assay revealed efficient to rank the oxidative potential of NPs. Indeed positive and negative control NPs induced expected oxidation level of cytochrome *c*. We successfully established a high-throughput assay of this test. By contrast neither CeO_2 nor TiO_2 and $BaSO_4$ NPs did induce clear oxidation of DTT whereas Mn_2O_3 NPs induced a dose-dependent oxidation of DTT. Besides, Mn_2O_3 NPs induced a dose dependent cytotoxicity and increase of antioxidant enzymes expression in bronchial epithelial cells. CeO_2 and TiO_2 NPs modulated slightly the antioxidant enzyme expression and induced moderate cytotoxicity whereas $BaSO_4$ had no or low effects. Furthermore non-cytotoxic concentrations of NPs lead to a dose-dependent increase of the IL-8 release in bronchial epithelial cells excepted for $BaSO_4$ NPs.
*Conclusion***:** Cytochrome *c* oxidation was the most sensitive acellular assay and could be used for high-throughput screening of the oxidative potential of NPs. The oxidative potential of the studied NPs correlated well with their cytotoxicity, pro-inflammatory response and antioxidant defense induced in bronchial epithelial cells. Mn_2O_3 NPs revealed good positive control and $BaSO_4$ negative control NPs. The highly used TiO_2 and CeO_2 NPs induced intermediate responses underlining the need to further study the pulmonary response to these important NPs.

N° O4c-4

NANOMATERIALS SOLUBILITY/BIODURABILITY AND REACTIVITY IN SYNTHETIC BIOLOGICAL FLUIDS AND CELL MEDIA

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To improve our understanding on the potential toxicological mechanisms of manufactured nanomaterials (NM) and particulate air-pollution in general, it may be necessary to improve our understanding of how NM react and dissolve in the biological systems or laboratory conditions under which the tests are conducted. To meet these questions, two different procedures were developed to investigate the oxidative and caustic reactivity of NM, as well as their dissolution and inferred biodurability in the lung and in different cell media. A 24-well SDR (Sensor Dish Reader) system was used for screening the 24-hour pH and O_2 reactivity and dissolution of MNs in various cell media and Gambles solution (GS); a synthetic lung lining fluid. A cell incubator was used to maintain the SDR test conditions (5% CO2, 37°C). A Temperature-pH-controlled Stirred flow-cell Batch Reactor (ATempH SBR) with online pH-control and monitoring of redox potential was used to test the ractivity and dissolution of NM at highly controlled lung lining (pH 7.4) and phagolysosomal (pH 4.5) pH conditions. The test conditions in the reactor were maintained by a thermostat (37°C) and bubbling of CO_2 adjusted air into the suspension. ATempH SBR tests were only made using GS and synthetic phagolysosomal fluid (PSF). In this presentation, the solubilities and reactivities of select TiO_2, synthetic amorphous silica, ZnO, and Ag NM using the two different test systems are described. Most of these MNs have weak causticity, but in SDR tests, the pH levels may vary at least 2 pH units due to whole-test system variations. Several MN showed considerable effects on the O_2 concentrations and the redox-potential in the ATempH-SBR. The dissolution of MN varies considerably with MN type, test media and pH-conditions. TiO_2 has very low solubility, but their inorganic coatings was observed to dissolve. Ag, amorphous silica, and ZnO show large variations in solubility depending on the test media and pH. Generally, the solubility is highest in the low pH PSF. Links between observed effects and physico-chemical properties will be investigated and presented.

N° O4c-5

EASY TO DIGEST ? A COMPREHENSIVE *IN VITRO* APPROACH TO MONITOR THE FATE OF ORALLY INGESTED NANOPARTICLES

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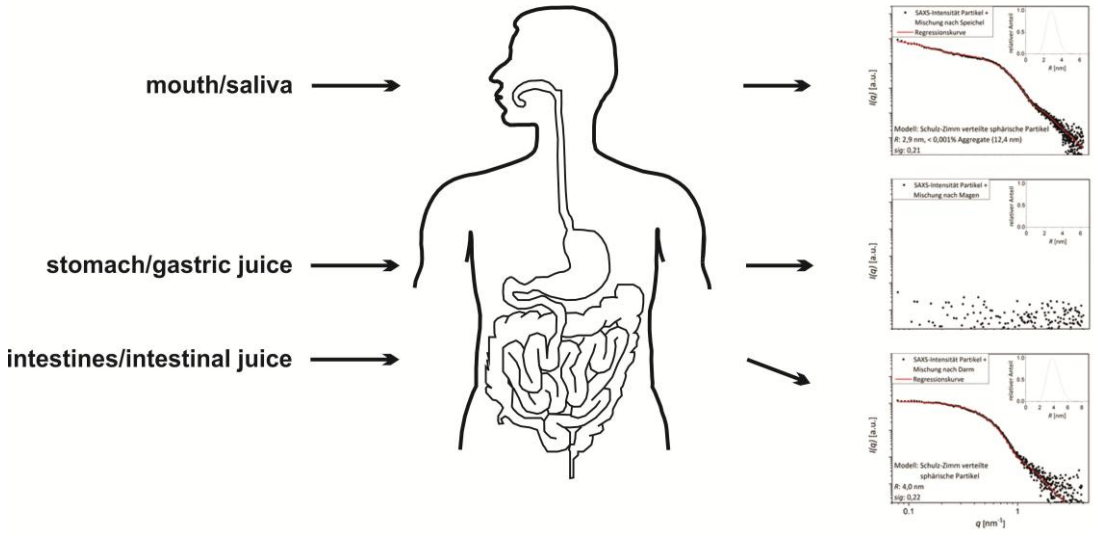
Determining physicochemical parameters of nanoparticles in complex media is a key issue for assigning nanospecific effects e.g. under physiological conditions.
In order to monitor in detail the effects of the physiologic environment of the human intestinal system on ingested nanoparticles, an *in vitro* approach is presented which accounts for the specific environments of mouth, stomach and bowel. The procedure is closely related to the german DIN 19738 which originally deals with the absorption availability of organic and inorganic pollutants from contaminated soil material and was adapted to the investigation of nanomaterials at BAM. Additionally, the influence of three representative nutrients as proteins in skimmed milk, olive oil and corn starch on the stability of nanoparticles during the process was monitored. For the experiments, well characterized reference silver nanoparticles were applied. These silver nanoparticles were closely monitored throughout the digestion process at all stages by means of Small-Angle X-ray Scattering (SAXS). SAXS is capable of yielding very accurate results regarding the size and shape of nanoparticles and is very sensible towards aggregation processes, too, despite the present matrix of salts, digestion enzymes and nutrients, so SAXS can be applied *in situ* without further sample preparation. Three silver nanoparticle reference materials were subdued to the comprehensive artificial digestion and the impact of the process on the differently stabilized particles is reported, answering the question whether or not ingested nanoparticles dissolve or aggregate or if they keep their *nano*form under certain circumstances.



N° O4d-1

STUDY OF LUNG LINING FLUID INTERACTIONS WITH NANOPARTICLES: TOWARDS MORE RELEVANT *IN VITRO* TOXICITY TESTS

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In the alveoli, where gas exchange takes place in the lung, inhaled particles first interact with the lung lining fluid, composed of phospholipidic vesicles and proteins1. Consequently, alveolar cells are not in contact with particles but more likely with particles surrounded by proteins corona or with particle-vesicles complexes, whose properties (stability, toxicity,…) would be different from those of the bare objects2.

Our goal is to study step by step the pathway, from inhalation to passage in the bloodstream, in order to understand what happens in each phase of this process.

To do so, four types of particles and three lung fluid models were characterized, in terms of size, zeta potential and stability. Mixtures of particles and lung fluid were prepared to detect significant interactions. Aggregates were observed both for positively and negatively charged particles, mixed with lung fluids, with or without proteins. Finally, interactions appear to be mainly driven by electrostatic forces, although weaker interactions, due to phospholipids, play a role.

Complexes between particles and lung fluid were observed in Phase Contrast Microscopy and Transmission Electron Microscopy (TEM), and the deciphering of their exact structure is being studied with TEM, Field Flow fractionation and Differential Centrifugal Sedimentation. Toxicity tests were performed on lung epithelial cells with particles and particles-lung fluid complexes. Current results indicate that the presence of lung fluid tends to decrease the particles toxicity.

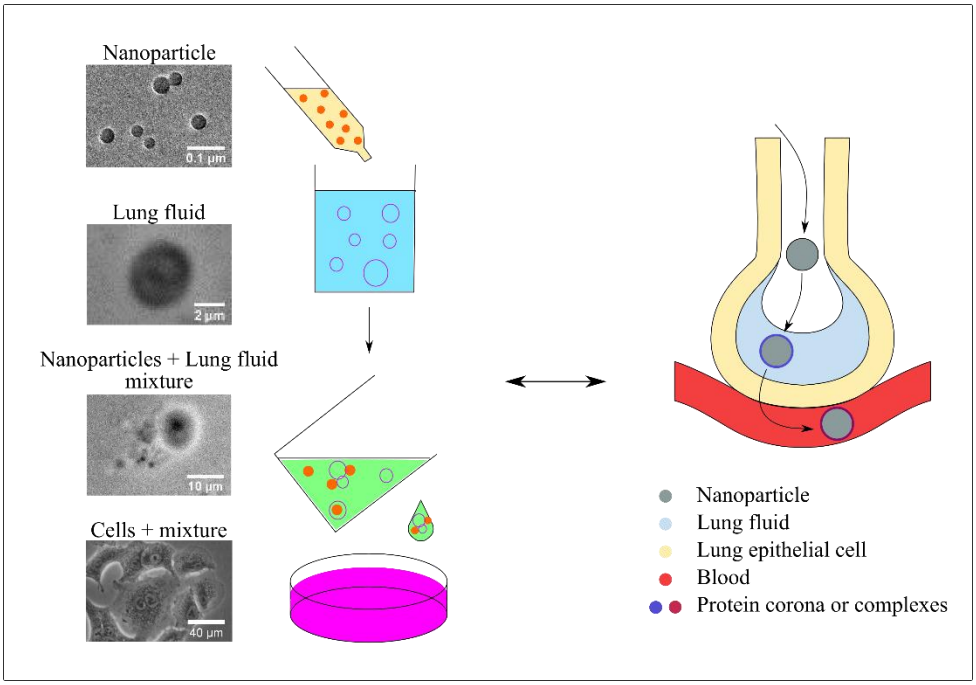

Figure 2 : Nanoparticles pathway from inhalation to passage in the bloodstream

1 J. Pérez-Gil, Biochimica et Biophysica Acta, 2008
2 Schleh et al. Particle and Fibre Toxicology, 2013

N° O4d-2

TOXICITY OF AG NANOPARTICLE AND INDUCTION OF AN INFLAMMATORY RESPONSE IN THE GI TRACT AND MEDIATION OF THIS TOXICITY BY ASSOCIATED BIO-FLUID COMPONENTS

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The advent of nanotechnology has led to the incorporation of nanoparticles into a wide and varying range of consumer products. Due to contact between nanomaterials, living organisms and the environment becoming more widespread and intimate, understandably concerns have been raised regarding the possible harm caused by this interaction. Silver (Ag) nanoparticles in particular are proving the most popular due to their antimicrobial activity. This study aims to investigate the toxicity of Ag nanoparticles on cells associated with the GI tract and to determine if their interaction with bio-fluid components associated with this GI tract can mediate this toxicity. Three bio-fluid components were studied cholic acid, deoxycholic acid and ursodeoxycholic acid (UDCA) all components of bile. Two cell lines were employed to correspond to regions of the body associated with these bio-fluid components, a liver cell (HepG-2) and a laryngeal HeLa derived epithelial (Hep2) cell line. First characterisation of Ag nanoparticles was carried out by dynamic light scattering (DLS), scanning electron microscopy (SEM), UV-vis spectroscopy and zeta potential analysis. Following this a number of toxicological assays were performed including Alamar Blue, MTT, ROS analysis and ELISA to investigate any inflammatory to determine toxicity and subsequent mediation by bio-fluid components. Results demonstrated the toxicity of Ag nanoparticles to the aforementioned cells lines and induction of an inflammatory response with mediation in some instances of this toxicity by bio-fluid components. It is clear from the data that it not only depends on the bio-fluid component itself but on the location in which it acts that determines mediation of toxicity.

N° O4d-3

CERIUM DIOXIDE NANOPARTICLES AFFECT *IN VITRO* FERTILIZATION IN MICE

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The fast development of nanotechnology and increasing environmental exposure to nanomaterials give rise to questions regarding the potential risks on human health. Due to their catalytic properties, cerium dioxide nanoparticles (CeO_2NP) are widely used, as diesel additive, as well as promising therapeutic in cancerology, yet scarce data are currently available on CeO_2NP's toxicity, and none on their reproductive toxicity. The Organization for Economic Cooperation and Development placed CeO_2NP on a priority list of nanomaterials requiring urgent evaluation. Our team recently disclosed the genotoxicity of CeO_2NP on mouse oocyte. Our objectives were to investigate CeO_2NP's genotoxicity on mouse spermatozoa, and their possible impact on murine *in vitro* fertilization (IVF).
A physico-chemical characterization of CeO_2NP was done, to assess aggregation and dissolution in culture medium. Comet assay was performed on mouse spermatozoa exposed *in vitro* to the following low concentrations for an hour: CeO_2NP 0,01mg/L, 0,001mg/L and the supernatant of CeO_2NP suspension at 0,01mg/L obtained by ultracentrifugation. We carried out mouse IVF, after female superovulation and fresh sperm collection, in culture media containing or not CeO_2NP. Fertilization rate was established the next day, from the number of two cells embryo over the total number of oocytes. Transmission Electron Microscopy (TEM) was performed on embryo and oocytes.
Comet assay showed significant genotoxicity of CeO_2NP on mouse spermatozoa, in the 3 conditions. DNA damage was quantified by % Tail DNA (mean ± standard error). DNA lesions were increased in spermatozoa exposed to CeO_2NP 0,01mg/L, 0,001mg/L and CeO_2NP 0,01mg/L supernatant (64,5 ± 0,67, 68,4 ± 0,68 and 35,3 ± 0,76 respectively), compared to negative control (18,3 ± 0,46), $p<0,0001$). Fertilization rates, assessed on 979 oocytes, were statistically decreased in oocytes exposed to CeO_2NP 0,01mg/L: 51,6% versus non-exposed: 62,4%, $p=0,0006$. TEM didn't show any nanoparticles in the gametes' cytoplasm, but their accumulation on oocytes zona pellucida and spermatozoa membranes.
This study demonstrated for the first time the impact of CeO_2NP on *in vitro* fertilization, as well as their genotoxicity on mouse spermatozoa at low NP concentration exposure.
We observed an increased genotoxicity of the suspension of nanoparticles compared to their supernatant. This result suggests a mechanism of genotoxicity involving the direct impact of CeO_2NP. Decreased fertilization rates may result from: 1) CeO_2NP's genotoxicity on gametes; 2) a mechanical effect, disrupting gamete interaction by accumulating along oocyte zona pellucida or spermatozoa membrane; 3) oxidative stress induced by CeO_2NP. Our results add new and important insight with regard to the reproductive toxicity of nanomaterials needing urgent evaluation, support several publications pointing out metal nanoparticles reprotoxicity and highlight the need for *in vivo* studies after low-dose exposure.

N° O4d-4

TREATMENT OF CELLS WITH NANOPARTICLES BOOSTS INTERCELLULAR COMMUNICATION

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Intercellular communication is a vital part of proper cellular functioning as it steers among others cellular proliferation, migration, differentiation and ultimately the correct build-up of tissues. Communication is carried out e.g. via soluble messenger molecules, physical interaction or the exchange of cellular membrane-bound material or vesicles.
While it was known that vesicular carriers transfer from cell-to-cell intracellular cargo like proteins and microRNAs, we have recently shown in normal rat kidney cells that nanoparticles (NPs) also are cargos for such a cell-to-cell transfer (Schoelermann *et al*., submitted). In addition, we observed that cell-to-cell transfer of vesicular cargo as such was increased when cells were treated with NPs.
We now report that this is a general trend in a wide range of different cell lines including chinese hamster ovary (CHO) cells, HeLa cells, the primary osteosarcoma cell line SaOs-2 as well as the human embryonic osteoblast cell line hFOB. The increase in intercellular communication accounted - depending on the cell line - between 1,3 to more than 3-fold and could be observed after treatment with different types of NPs.
Our assay, which is based on differential fluorescent labelling and flow cytometry, will be introduced and possible reasons as well as implications for the observed NP-induced increased communication will be discussed.

N° O4d-5

TITANIUM DIOXIDE NANOPARTICLES TOXICOLOGY: TOWARDS MORE PHYSIOLOGICAL IN VITRO EXPOSURE MODELS?

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Most studies dedicated to the investigation of *in vitro* titanium dioxide nanoparticles (TiO_2-NPs) inhalation genotoxicity use exposure models where cells are exposed to NPs suspensions, at high concentration. The relevance of such models is debatable because i) upon inhalation, lung cells are exposed to an aerosol of NPs rather than a liquid suspension of NPs and ii) the exposure dose is much higher than physiological exposure doses, even in a worst-case scenario (Paur 2011).

Based on our previous data, obtained with a classical exposure setup (Jugan 2012), the aim of this study was to evaluate TiO_2 NPs genotoxicity in more relevant exposure conditions.

We first used a chronic exposure model, in which A549 cells were exposed to 1-50 µg/mL of NPs, in suspension, during up to 2 months. We did not detect any cell mortality in these exposure conditions, but we did observe a time and dose-dependent increase of NPs accumulation in cells as well as oxidative stress response. Exposure led to primary DNA lesions, assessed using the Comet assay (alkaline and fpg- versions) and immunostaining of 53BP1 foci (representative of double strand DNA breaks). However, the micronucleus test, indicative of aneupleuoidy-clastogenicity, turned out negative.

Exposure during 2 months to 2.5 or 50 µg/mL of TiO_2-NPs caused significant modulation of expression of 27 proteins, involved in pathways including protein folding, intracellular trafficking, or inflammation.

Our second exposure scenario was Air-Liquid Interface (ALI) exposure to an aerosol of TiO_2-NPs, in collaboration with the Karlsruhe Institute of Technology (Germany). Cells were grown on Transwell® inserts, then exposed on their apical side to a NPs aerosol after withdrawing the apical cell culture medium. The aerosol of NPs was characterized in terms of size distribution, homogeneity and deposited dose.

The first results showed that, for a 4-hour exposure, the NPs aerosol was not cytotoxic. Genotoxic events were observed, through both comet assay (alkaline and fpg- versions) and 53BP1 foci measurement.

Taken together, these results show that even in realistic exposure scenarios TiO_2-NPs induce oxidative stress and DNA damage in lung cells.

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N° O4d-6

AN APPROACH TO EVALUATE WHICH *IN VITRO* MODEL AND EXPOSURE METHOD IS MORE PREDICTIVE FOR *IN VIVO* BIOLOGICAL RESPONSES

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The growing utilization of nanomaterials (NMs) in nanotechnology products lead to a potential increase of exposure, thus raising concerns about workers and public's health risks. The major route of exposure is inhalation, but so far occupational and environmental atmosphere are not well characterized in terms of NMs. Despite the lack of epidemiological data on the relation between exposure to NMs and human health effects, their potential toxicity has been studied on cell cultures and animal models. Among these studies, most results are from *in vitro* experimentations due to the difficulty to perform *in vivo* studies for the enormous number of existing NMs and the necessity to reduce number of animals used in experimentations (3R rules). Nevertheless, results from animal experimentations remain the most reliable. Even if, pushed by the necessity to reduce the number of animals used in experimentation, new *in vitro* models and exposure methods are and have been developed, suggesting the more and more relevant alternatives to animal experimentation.
Thus, many studies show that newly developed co-culture or 3D *in vitro* models have different nanotoxicity responses compared to classical mono-culture models. Moreover, studies using new devices allowing exposure of cells to aerosols of NMs show different nanotoxicity responses compared to exposure to suspensions of NMs. However, which of these models and exposure methods is more predictive for *in vivo* responses is yet to be defined.
In order to better define which *in vitro* model and which exposure method is more predictive for *in vivo* pulmonary nanotoxicity data, three different methodologies will be implemented.

- Alveolar epithelial cell line A549 in monoculture or in coculture with the alveolar macrophages cell line THP-1 will be exposed to a suspension of NM in culture media until 24h.

- Same *in vitro* culture models will be exposed at the Air Liquid Interface (ALI) to NMs aerosols with the VitroCell® system, for 3h and then left at incubator until the remaining 21h.

- Rats will be exposed in acute (1day) or in repeated manner (5 and 28 days), to NMs aerosol into a nose-only inhalation system (HCT®), and euthanasia will be performed just after and 35 days after exposure.

For each methodology, 5 metallic NMs (TiO2 NM105, NM100, NM101; CeO2 NM212; BasSO4 NM220) possessing different physico-chemicals and toxicological properties will be used. Biological endpoints including cytotoxicity, inflammation, oxidative stress and genotoxicity will be assessed. Then, the results will be expressed in term of lowest dose causing biological effect for each endpoint, each NM and each methodology used, to allow comparisons between *in vitro* and *in vivo* data.
As first part of the project, NMs aerosols generation assays for *in vivo* and *in vitro* exposures have been performed. Thus, a common generator, the nebulizer AGK 2000 PALAS® has been chosen for both studies, because it allows a stable and repeatable generation with high number of nanoparticles in aerosols. Also, a VitroCell® system, a device allowing cells exposure to NMs aerosol in ALI has been set up with TiO_2 nanoparticles and first *in vitro* ALI and submerged exposures results have been obtained. These results will be presented during Nanosafe conference.

N° O4e-1

HOW DO OXIDE AND 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 (Piccione et al., 2012), medicine (Wang et al., 2009), products of personal use (Calzolai et al., 2012), and food (Napierska et al., 2012). However, there are health concerns regarding the use of ENMs (Oberdörster, 2001). 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 key physicochemical parameters of ENM dispersions, concentration and particle-size distribution, was studied using an in vitro biological assessment. These parameters were assessed in a set of cell lines: A549, IHK, and ZS95 incubated at different times. A set of ENMs were selected for the assessment: SiO2 and TiO2 (part of the OECD list of reference nanomaterials), and SiC and TiC (two materials widely used in the metallurgy industry). ENM concentration was quantified with PIXE (Particle-Induced X-ray Emission) and its particle size distribution was measured by CLS (Centrifuge Liquid Sedimentation). The contribution of the cell lines was elucidated by performing the same set of measurements only on cell culture media (without cell lines). Preliminary results show a correlation between the ENM physicochemical properties and the cell media used (Lozano et al., 2013). The validity of the current assumption, that a given dose equals the sedimented dose, will be discussed in light of the findings.

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N° O4e-2

CHARACTERIZATION OF THE OXIDATIVE POTENTIAL OF NANOMATERIALS

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Toxicological studies have shown that a comprehensive knowledge of manufactured nanomaterials (MNM) and their reactions with organisms and the environment is required to determine their adverse effects. Certain characteristics as their size, surface properties and their ability to form reactive oxygen species (ROS), are promising metrics to predict harmful outcomes from the exposure to MNM. The aim of the NanOxiMet project is to group MNM by a combination of the analysis of physico-chemical characteristics and in vitro toxicity tests in order to be used as valid tools to predict the pathogenicity of MNM. Therefore a range of different nanoparticles were chosen, including (noble) metals, metal oxides and carbonaceous particles, obtained from OECD or commercially available: ZnO, CeO_2, $BaSO_4$, coated and uncoated TiO_2, Cu, Fe_2O_3, carbon black (Printex 90, Lamp black), Al_2O_3. MNM were suspended in water and dispersed by indirect sonication. Characterization of particles sizes was done by measuring their hydrodynamic diameter which ranged from 180 to 680 nm. Since inhalation is one primary exposure route we studied the effects of MNM on NCI-H292 cells, a human bronchial epithelial cell line, and on THP-1 cells differentiated into macrophages. Cellular viability was assessed by WST-1 assay on cells exposed to MNM at 5, 10, 20 and 40 µg/cm^2 for 4 and 24 hours. A high cellular toxicity was observed for ZnO and Cu exposed cells, for other MNM cellular toxicity was less pronounced. Intrinsic oxidant generation capacity of MNM suspensions was investigated through consumption of antioxidants in a synthetic respiratory tract lining fluid composed of 200 µM of uric acid (UA), ascorbic acid (AA) and glutathione (GSH) by incubation with MNM at 16 - 256 µg/ml for 4 hours at 37°C. HPLC quantification showed that incubation with Cu resulted in complete depletion of AA as well as GSH and Printex 90 induced partial consumption of the same antioxidants. These same particles exhibited also an oxidation of dithiothreitol (DTT). DNA strand break of bacteriophage φX174 was induced by Cu MNM, whereas for other particles DNA damage was evident only in presence of H_2O_2 (ZnO > TiO_2 NM104 > Fe_2O_3), which can be explained by Fenton reaction producing ROS.The results obtained to date indicate that ZnO nanoparticle cytotoxicity is not related to an increased oxidative potential and that Cu nanoparticles have a high reactivity, probably being the underlying mechanism of their cytotoxicity. Other acellular assays are currently under investigation to further characterize the intrinsic oxidant generation capacity of the studied MNMs.
“This study in included in the NanOxiMet project (www.nanoximet.eu) that is supported by the French National Research Agency (ANR) and the German Federal Ministry of Education and Research (BMBF), under the frame of SIINN, the ERA-NET for a Safe Implementation of Innovative Nanoscience and Nanotechnology.”

N° O4e-3

FATE OF METALLIC NANOPARTICLES IN CELLULAR MODELS: DISSOLUTION, SPECIATION AND COMPLEXATION *IN CELLULO* PROBED BY SYNCHROTRON-BASED TECHNIQUES

Giulia Veronesi[1,2], Emilie Brun[3], Thomas Gallon[1], Martine Cuillel[1], Peggy Charbonnier[1], Françoise Rollin-Genetet[4], Claude Vidaud[4], Pascale Delangle[5], Catherine Aude-Garcia[1], Thierry Rabilloud[1], Elisabeth Mintz[1], Isabelle Michaud-Soret[1], Marie Carrière[5] (1) a) CEA, Laboratoire de Chimie et Biologie des Métaux (LCBM), Grenoble, France b) CNRS, LCBM, F-38000 Grenoble, France c) Univ. Grenoble Alpes, LCBM, Grenoble, France. (3) UMR3299 CEA-CNRS SIS2M/LSDRM CEA Saclay, 91191 Gif-sur-Yvette, France (4) CEA/DSV/iBEB/SBTN, BP 17171, 30207 Bagnols sur Cèze, France (5) a) Université Grenoble Alpes, INAC, SCIB, F-38000, Grenoble, France; b) CEA, INAC, SCIB, F-38054 Grenoble, France.The widespread use of nanoparticles (NP) in textiles and food industry fosters the need to assess their toxicity in humans. The complex phenomena ruling the toxicity of NP are intimately related to their physical-chemical properties in cells and tissues, and to the transformations they undergo once incorporated in their biological hosts. We made use of synchrotron-based techniques, namely X-Ray Fluorescence microscopy (µXRF) and X-ray Absorption Spectroscopy (XAS) to investigate the fate of metallic NP upon uptake by cellular models: these techniques allow retrieving subcellular distribution and speciation *in cellulo* of the metals, and to highlight their complexation with the molecular groups from cell components. When the NP are reputed inert, like titanium dioxide (TiO_2) NP, it is crucial to probe their solubility in physiological conditions, especially in presence of very harsh media like gastric fluids or inside intracellular compartments; when the solubility of the NP is well known, as for silver (Ag) NP, a fundamental challenge consists in determining the fraction of metal ions entering cells upon exposure and to correlate it to cytotoxicity. Our approach aims at answering these questions.TiO_2 micro- and nanoparticles are used for many applications, and particularly in food packaging and additives, humans are therefore exposed through ingestion. We investigated the fate of TiO_2 NP in a model of gastrointestinal (GI) epithelium by means of µXRF and XAS, in order to ascertain if translocation through the GI barrier occurs; we found that rutile [1] and anatase [2] TiO_2 NP tend to accumulate in epithelial cells without undergoing dissolution and we measured the interatomic distances in rutile crystal structure *in cellulo* [1].

Ag NP are widely used in consumer goods for their antibacterial properties; following exposure, they accumulate mainly in the liver. By means of XAS we assessed the complexation of Ag(I) from Ag NP in human hepatocytes HepG2 cell line and in primary murine macrophages: our data argue for the release of Ag(I) from NP upon 6- to 24-hours exposure followed by recombination with thiol groups in the cells. Moreover, we investigated the influence of the NP coating on the dissolution kinetics.
More in general, this approach based on the combination of µXRF and XAS is the only one capable to provide with crucial information for the assessment of metallic NP toxicity, like their distribution, dissolution and complexation in cells.

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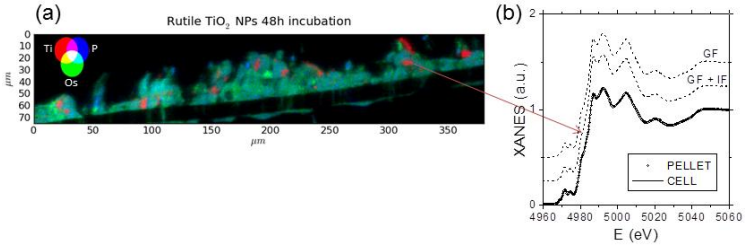

Figure: (a) µXRF *image of Ti (Red) Os (Green, incorporated through the fixation process) and P (Blue) in a model of GI epithelium exposed for 48 hours to its apical pole to TiO_2 NP diluted in complete medium. (b) XAS spectra of rutile TiO_2 NP in different conditions: raw (empty circles), matured in gastric fluid (dashed, GF), matured in gastric and then intestinal fluids (dashed, GF+GI) and incorporated in GI cells.*

N° O4e-4

THE INFLUENCE OF SIZE, TIME AND DOSE ON THE TRANSLOCATION OF GOLD NANOPARTICLES ACROSS MOUSE AND HUMAN ALVEOLAR EPITHELIAL CELL MONOLAYERS

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Introduction: Inhalation of nanoparticles (NPs) is considered to be one of the most important routes for human exposure. Hence, much research is devoted on the uptake mechanism of NPs by lung cells and possible cellular responses. However, to date very little is known about the translocation kinetics of NPs across the air-blood barrier. To determine the translocation kinetics of NPs and possible interspecies differences human and mouse alveolar epithelial cell monolayers were exposed to NPs of different size and dose at the air-liquid interface (ALI). For this purpose, we used gold NPs a as model particles, due to their low toxicity and high stability in biological systems.
Methods: A549 (human alveolar type II) and MLE-12 (mouse alveolar type II) cell monolayers were cultured on porous membranes (3 µm pore sizes) for 7 days and 3.5 days under submerged conditions and 1 day at the ALI before particle exposures, respectively. The cell were exposed to different doses (25, 50, 100, 150, 200 ng/cm^2) and sizes (2, 7, 17, 46, 80 nm) of citrate coated gold NPs or an ionic gold solution using the air-liquid interface cell exposure system (ALICE), which was already previously presented as an effective way to mimic inhalation exposure [1]. The distribution of the gold NPs between the surfactant layer, the cell monolayer and the basolateral medium after different time points (0, 2, 8, 24, 48 or 72 hours after exposure) was measured using ICP-MS. From these results the translocation fractions of the different sizes and doses of NPs and time points were determined. Furthermore, to be able to compare the obtained translocation fractions to available *in-vivo* data, we used our recently presented physiologically based pharmacokinetic (PBPK) model for NPs [2].
Results: There is no significant difference in the translocation kinetics between A549 and MLE-12 cells. The translocated fraction within 24 hours is independent of the applied dose. Only above 150 ng/cm^2 we can observe a decrease in the relative amount of translocated gold NPs. From our results we can also observe a fast translocation of NPs within the first eight hours and a much slower translocation that becomes dominant after 24 hours, which indicates that the NPs use different pathways to cross the cell monolayer. The most interesting finding is, however, the influence of size on the translocation fraction of NPs through the cellular monolayer, which is inversely proportional to their size. While only 2-4% of the particles larger than 17 nm cross the monolayer within 24 hours, the translocation fraction of the 2 nm particles is around 60%, which is close to the translocation fraction of ionic gold (*ca.* 80%). The validity of these results was supported by comparison of these findings to available *in-vivo* data [3]. To this end we used a PBPK model, which allowed to compare *in-vitro* to *in-vivo* translocation fractions and also to successfully predict the biodistribution of translocated NPs.

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[2] Bachler et al. (2014) Using physiologically based pharmacokinetic (PBPK) modeling for dietary risk assessment of titanium dioxide (TiO_2) nanoparticles, Nanotoxicology, early online
[3] Kreyling et al. (2014) Air-blood barrier translocation of tracheally instilled gold nanoparticles inversely depends on particle size, ACS Nano, 8(1):222-233.

N° O4e-5

COMPARISON OF *IN VITRO* CYTOTOXICITY AND OXIDATIVE STRESS OF POLY(PROPYLENE IMINE) AND POLY(AMIDO AMINE) DENDRITIC NANOPARTICLES.

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The *in vitro* cytotoxic and intracellular oxidative stress responses to exposure to poly(propylene imine) (PPI) dendritic nanoparticles of varying generation were assessed in an immortal non-cancerous human keratinocyte (HaCaT) cell line and were also compared to those previously documented for poly(amido amine) (PAMAM) dendritic nanoparticles. Exposure to PPI dendrimer generations 1-5, having 4, 8, 16, 32 and 64 primary surface amino groups respectively, was examined and the results were compared to similar exposures to PAMAM generations 4-6, having 64, 128 and 256 primary surface amino groups respectively. The size distribution of the nanoparticles was examined using both Dynamic Light Scattering and Atomic Force Microscopy. The MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay was used to measure cell viability and oxidative stress was monitored according to changes in intracellular reactive oxygen species (ROS), as measured using the Carboxy-H2DCFDA dye assay, as a function of generation, dose and exposure time. A generation and dose dependent cytotoxic response was observed, increasing according to generation and therefore number of surface amino groups. A biphasic increase in ROS was observed for 3 - 5 PPI generations, the initial increase showing a maximum within 3-6 hours, before a secondary increase up to the maximum exposure time of 24hrs. In contrast, PPI G1 and G2 had a monophasic ROS generation where maximum ROS was observed at 24hrs. This may indicate that the smaller dendrimers are internalized by an alternative mechanism, as the initial ROS has been attributed to endocytosis. Confocal fluorescence microscopy demonstrated that the secondary phase of ROS was localized in the cell mitochondria, similar to previous observations for exposure to PAMAM dendrimers. Although a comparison of the cytotoxic response of the G5 PPI and G4 PAMAM dendrimer indicates that the PPI dendrimer with the same number of surface amino groups elicits a significantly higher cytotoxic response, for PPI G3 – G5 and PAMAM dendrimer series, the cytotoxic response is well correlated with the early stage oxidative stress levels, indicating a common mechanism of response. From the results it is clear that PPI G1 and G2 stray from the active uptake, the most widely accepted mechanism of internalization of dendrimers. This may indicate that PPI G1 and G2 have crossed the threshold between particle and molecule, characterised by active and passive uptake mechanisms, respectively.

N° O4f-1

THE NEED FOR PHYSIOLOGICALLY-BASED MODELS TO PREDICT NANOPARTICLE BIODISTRIBUTION

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So-called physiologically-based pharmacokinetic (PBPK) models have long been extensively use to understand and predict the pharmacokinetic behavior including the absorption, distribution, metabolism and excretion (ADME) of chemicals in the body. The aim of this presentation is to highlight the usefulness of and need for PBPK modeling of nanoparticle (NP) biodistribution. To date, only a few models of this kind have been published.

PBPK modeling is a mathematical description of the ADME behavior. It is implemented by solving a set of differential equations over time, thereby describing the time course of the amount or concentration of the parent chemical and its metabolite(s) in one or several body compartments. A major reason for the PBPK approach is the target dose concept, i.e., that the toxic effect (or NP) only arises when the chemical or its metabolite(s) reach specific targets within the body and a quantitative relationship between target dose and toxic effect can be expected.

One important feature of nano PBPK models is that they allow extrapolation from dose *in vitro* to dose *in vivo*. In addition, factors known or expected to influence the relationship between the external dose and the target dose may be accounted for. Thereby, some of the uncertain factors associated with toxicological risk assessment may be reduced. Important factors include e.g.: route of exposure, exposure pattern, exposure duration, physical exercise, and species differences in size, physiology, and metabolism.

NPs, being suspended particles, differ from dissolved chemicals in that they diffuse much more slowly and do not easily pass through cell membranes. Thus, diffusion, endocytosis and phagocytosis (rather than blood flow) tend to become rate-limiting for NPs. The key factors when modeling dissolved chemicals – organ volumes, blood flows, partition coefficients, metabolic capacity etc. – are well understood and a wealth of data on relevant anatomical, physiological and biochemical parameters have been collected for various species, including humans, over the years. I contrast, the key factors for NP biodistribution are less well defined and the corresponding parameters, such as the behavior and number of phagocytizing cells in different tissues, are largely unknown.

PBPK models may also be used to strengthen experimental results by linking data from different experiments in a uniform model. One might, for example, use a single generic model to explain NP biodistribution after different routes of administration or to describe uniformly the biodistribution of various NP types. When the initial attempt fails, a modified or alternative – but still biologically relevant - model may be developed and tested. Thus, modeling is often hypothesis generating. Once the model is established, it may be expanded to facilitate extrapolation, e.g. other types of NPs or other exposure routes.

We have modified and expanded our previous PBPK model (1) for intravenously injected pegylated polyacrylamide (PAA) NPs in rats to three additional NPs; uncoated PAA, gold and titanium dioxide. The modified model (2) adequately describes the ADME of all four NPs and confirms that phagocytosis is a critical process. The clearance from blood to tissue is fast during the first hours after injection, therefore frequent sampling during this period is needed to better understand the key processes. On the other hand, long post-dosing follow-up is also important to account for bioaccumulation. We also note that the dose has a major impact on the biodistribution. Another observation is that the limit of detection needs to be sufficiently low to address potential uptake in compartments with assumed barriers, e.g. the brain and the fetus. More detailed *in vivo* studies are required for improved PBPK modeling, including data on: dose, recovery, particle size and size distribution, and integrity/stability of the NP, more frequent sampling especially early post-dosing, longer follow-up, more tissues analyzed, more samples per tissue, and individual data on body and organ weights.

1. *Li D et al. Nanotoxicology 8 S1 (2014) 128–137.*
2. *Johanson G et al. BfR-Workshop on Biokinetics and Environmental Fate. Berlin (2014).*

N° O4f-2

EFFECTS ON THE NERVOUS SYSTEM OF EXPOSURE TO ENIGNEEREDNANOMATERIALS – AN OVERVIEW

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There are certain concerns regarding the safety for the environment and human health from the use of engineered nanomaterials (ENMs) which leads to unintended exposures, as opposed to the use of ENMs for medical purposes. This overview focuses on the unintended human exposure of ENMs. In particular, possible effects on the functions or processes in the brain are discussed and an attempt to assess risks is performed.

Animal experiments have shown that investigated ENMs (metallic nanoparticles, quantum dots, carbon nanotubes) can translocate to the brain from different entry points (skin, blood, respiratory pathways). After inhalation or instillation into parts of the respiratory tract, a very small fraction of the inhaled or instilled ENMs reaches the blood or secondary organs, including the CNS, at a low translocation rate. Experimental in vivo and in vitro studies have shown that several types of ENMs can have various biological effects in the nervous system. Some of these effects could also imply that ENMs can cause hazards, both acutely and in the long term. The relevance of these data for risk assessment is far from clear. There are at present very few data on exposure of the general public to either acute high dose exposure or on chronic exposure to low levels of air-borne ENMs. It is furthermore unlikely that acute high dose exposures would occur. The risk from such exposures for damaging CNS effects is thus probably very low, irrespective of any biological effects that ENMs could have.

The situation is more complicated regarding chronic exposures, at low doses. We do not have exposure data for the general public regarding ENMs. Although translocation to the brain via respiratory organs and the circulation appears to be very low, there remains a possibility that chronic exposures, and/or biopersistent ENMs, can influence processes within the brain that are triggering or aggravating pathological processes.
In general, the present state of knowledge is unsatisfactory for a proper risk assessment in this area. Crucial deficits include lack of exposure data, the absence of a proper dose concept, and that studies often fail in adequate description of the investigated ENMs.

N° O4f-3

CYTO- AND GENOTOXICITY OF SILVER NANOPARTICLES IN HUMAN KERATINOCYTES: INFULENCE OF SURFACE COATING

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Silver nanoparticles (AgNPs) are one of the most commercialized nanomaterials, extensively used in various medical and general applications, mostly due to their characteristics as potent antimicrobial agents. Their use in large scale raises the concern about the potential toxic effects to humans and the environment. In spite of the increased number of studies undertaken in the last years in order to evaluate their toxicity, there is no complete understanding of the mechanisms associated with their toxicity, mostly due to the high variability in testing conditions, mostly related with size, coatings and stabilization. In this study we aimed to investigate the cyto and genotoxicity of AgNPs using the human keratinocyte cell line HaCaT as an in vitro model, as skin is a major entry route of AgNPs into the body. HaCaT cells were exposed to citrate-and PEG coated AgNPs of 30 nm, for 24 and 48h. Results showed that citrate coated AgNPs induced a higher reduction in cell viability than PEG coated. Also, both citrate- and PEG-coated AgNPs induced oxidative stress in HaCaT cells due to increased ROS levels and impaired antioxidant capacity, an effect more visible for citrate coated AgNPs. Citrate- and PEG-coated AgNPs also affected differently cell cycle progression, with citrate-coated AgNPs inducing an arrest at G2, and PEG-coated AgNPs inducing mainly S phase delay. Our results also show that AgNPs induce apoptosis mostly at the 24h period. Furthermore, transcriptome responses were supportive of the alterations observed in cell cycle and apoptosis. In conclusion our work shows that surface coating influences the toxicity profile of AgNPs.

Acknowledgments
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N° O4f-4

NANO-TiO_2 MODULATES THE DERMAL SENSITIZATION POTENCY OF DNCB AFTER TOPICAL EXPOSURE

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Taking the increasing use of engineered nanoparticles (ENPs) in industrial and consumer products, there is an urgent need for assessing their possible skin sensitizing potential as well as for their impact on the skin sensitization caused by chemicals. In this study, we determined the ability of different ENPs (TiO_2, Ag and SiO_2) and aged paint particles containing ENPs to modulate the dermal sensitization by a known potent dermal sensitizer (dinitrochlorobenzene) using a variant of the local lymph node assay. The fur of BALB/c mice in the area around and between the ears was cut with scissors one day prior to topical exposure to ENPs (0.4, 4 or 40 mg/ml), paint particles containing ENPs (4 mg/ml) or vehicle (day 0). On days 1, 2 and 3, the mice received dermal applications on the back of both ears of 2,4-dinitrochlorobenzene (DNCB) or vehicle (acetone olive oil (AOO)). The stimulation index (SI) was calculated on day 6. In AOO treated control mice, a prior exposure to TiO_2, Ag or SiO_2 ENPs, or aged paint particles did not influence the SI. When TiO_2 ENPs were applied prior to DNCB sensitization, we found an increasing SI using 4 mg/ml TiO_2 ENPs (medium dose), compared to vehicle exposed mice prior to DNCB sensitization. Topical exposure to Ag or SiO_2 ENPs, or aged paint particles prior to DNCB sensitization did not significantly influence the SI compared to DNCB sensitized control mice. In conclusion, we have demonstrated that topical exposure to TiO_2 ENPs increases the chemical-induced *in vivo* dermal sensitization.
Acknowledgements: This work is part of the EU FP7 project: NanoHouse (ID 207816*)*

N° PL5

NANOTECHNOLOGY, GLOBAL DEVELOPMENT IN THE FRAME OF ENVIRONMENTAL RISK FORCASTING. A NECESSITY OF INTERDISCIPLINARY RESEARCH

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Nanotechnology is no more an emerging technology. More than 2000 nanoproducts are already on the market (Nanowerk 2012). The variety of nanoproducts is large going from cosmetics to electronic, paints to food and health. The complexity of these nanoproducts increases in terms of structural and dynamic complexity: (1) passive nanostructures, (2) active nanostructures, (3) nanosystems, and (4) molecular nanosystems (M.C Roco. 2011). To date, new nanomaterials that are the object of intense researches are the graphene and the core-shell nanoparticles used as vectors for biological applications.

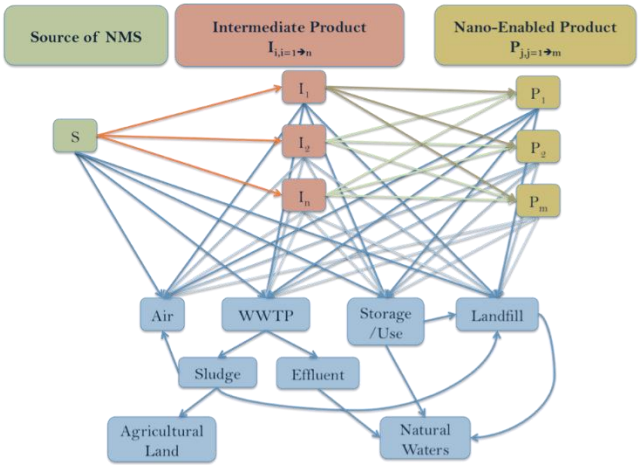
Nevertheless, the nanotechnologies are at the heart of societal interest and also fear since more than 15 years (*e.g.* ETC, and other citizen groups). One of the first reasons of this fear is the size of the nanoparticles that is seen as the parameter that could induce health and environmental problems. In this regards, governments and politic organizations have decided to fund national and European scientific projects about the risk assessment of nanotechnology in order to decide normalization politics through OECD countries. For instance, regulation is presently evolving to take into account nanoscale in products (for instance REACH).

Compared to nano-toxicity, nano-eco-toxicity is seen as a new discipline. While, many biologists considered that nanomaterials have the same environmental behavior as 'classic' pollutants as organic molecules or metals, physicists, chemists, geologists, physical chemists considered the structure-size relationship has been heart of their scientific and technological interests. The challenge in nano-eco-toxicity is to work with an interdisciplinary approach with the weak information available concerning the quantities of nanomaterial which could be released all along the life-cyle and taking into account the complexity of the environmental matrices. This requires to evaluate the exposure, (bio) transformations, bio (distribution) and biological effects of the nanomaterials at environmentally relevant doses and on the long term.

The complexity of assessing the environmental risk of nanomaterials is due to the fact that the exposure is the result of:

- the transport and transfer of nano particles for instance from soil to plants or from water to food chain
-the interactions of the nanomaterials between them or with the environment (e.g. interactions with other particles, organic molecules, cellular membranes) leading to the formation or not of aggregates (homo- or hetero-aggregates) which can impact the initial reactivity of the primary nanoparticles .- the transformation of the nanoparticles due to reduction, oxidation, dissolution leading to the formation of new mineral phases which could have the different reactivity compared to the primary nanoparticles.
- the behavior of the coating associated with the synthesis or the interactions wit environmental organic molecules which can affect the aggregation states and the reactivity and consequently the exposure and impacts of nanomaterials.

This must be evaluated along the flow of Nms through the value chain and life cycle (M.Wiesner and JY Bottero. CRAS Physics 2011).


Source of NMS
Intermediate Product
$I_{i,i=1\rightarrow n}$
Nano-Enabled Product
$P_{j,j=1\rightarrow m}$
S
I_1
I_2
I_n
P_1
P_2
P_m
Air
WWTP
Storage /Use
Landfill
Sludge
Effluent
Agricultural Land
Natural Waters

N° O5a-1

IMOGOLITES AS A MODEL FOR THE STUDY OF NANOPARTICLE'S ECOTOXICITY TOWARD *PSEUDOMONAS BRASSICACEARUM*

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Nanoparticles have unique properties compared to their micrometric counterparts. Because of their industrial use and their release into the environment, regulation of manufactured nanoparticles and assessing their environmental impact becomes an important issue. However, only few studies of (eco) toxicology linked the physico-chemical properties of nanoparticles mechanisms to the toxicity or the stress they induce. Moreover, no clear conclusions can be drawn at present because of the variability of nanoparticles used in studies especially in term of preparation methods, structure integrity and presence of impurities. In this context, this study aims to connect the physico-chemical properties of inorganic nanotubes to their ecotoxicity.
The nanoparticles we used in this study are imogolites. They are analogous of natural nanotubes, structured aluminosilicate at short distance and results from the alteration of glass and volcanic ash. Since 1977, various synthetic protocols proposed in the literature have led to a better understanding of the structure and reactivity of these aluminosilicates and isostructural analogues of alumino-germanates. These studies allowed us to control one by one their structural parameters (length, diameter, single / double walls, structural defects) and thus their physicochemical properties.
These nanotubes with varying properties were added to cultures of *Pseudomonas brassicacearum*, soil bacteria of the rhizosphere of *Brassica napus* and *Arabidopsis thaliana*. In order to finely investigate the stress and the toxicities induced by the presence of imogolites in bacterial cultures, each type of imogolite was characterized before and after contact with the bacteria. In addition, metabolic mechanisms induced by the imogolites presence were discussed at the cellular level by monitoring bacterial growth and the induced oxidative stress.
We were able to identify several mechanisms modulating the toxicity of imogolite to *Pseudomonas brassicacearum*. The tube length played an important role where the shortest tubes reduced bacterial growth when the longest have no effect on bacterial growing. But other criteria also appear to modulate these interactions. Indeed, the crystallinity of imogolites, including the presence of vacant sites on the surface, appears to modulate the bioavailability and thus the deficiency of nutrients in the culture media.
Thus this study shows the broad difference responses of bacterial communities to nanoparticles presenting the same chemical composition but several varying physico-chemical parameters, and vice versa. These results could be a promising step for giving clues for safer nanoparticle engineering.

N° O5a-2

RELATIONSHIPS BETWEEN NANO-DESIGN OF CERIA NANOPARTICLES AND THEIR IMPACT ON A TERRESTRIAL SOIL-MICROBE-PLANT ECOSYSTEM

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Over the last few years, there has been increasing interest in nanoscale cerium oxide *e.g.* for oxygen storage capacities, and optical and catalytic properties. Nano-CeO_2 is widely used in commercial products and industrial applications. Unfortunately, some of them are dispersive in nature. As with the introduction of most new technologies, there are some concerns regarding potential risks of exposure and interactions with the environment.
During the last years, some works have investigated the environmental impacts of ceria nanoparticles. However, many of these results are difficult to extrapolate to ecosystems, as to date, research on the environmental impact of nano-CeO_2 has largely focused on unrealistic high concentrations of nanomaterials. Based on the sole use in fuels, environmental concentrations of nano-CeO_2 are predicted in the range 0.28 - 1.12 mg/kg in soils. Furthermore, data are limited regarding the exposure mechanism, and do not highlight the impact on the plant microbiome.
To this purpose, we implemented terrestrial ecosystems to explore the response of rapeseed (*Brassica napus*) to nano-CeO_2 added in soil at 1 mg/Kg, and to analyze the impact on microbial community structure. Besides, we investigated the relationships between nano-design and impacts on soil-plant-microbe ecosystem using pristine or citrate-coated nano-CeO_2, with different average particle sizes and surface reactivity.
The presentation will focus on the relationships between physicochemical properties and impact of nano-CeO_2 on plant response to stress, based on enzymatic activities (catalase, peroxidase, ascorbate peroxidase…), alteration of micronutrients uptake by the plant and on the fluctuations of microbial communities.

N° O5a-3

ENVIRONMENTAL TRANSFORMATIONS OF SILVER NANOPARTICLES: IMPACTS ON STABILITY, BIOAVAILABILITY AND TOXICITY.

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The development of nanotechnologies has led to increasing concerns about the potential risk of manufactured nanoparticles in natural systems. Characterizing the environmental transformations of such nanoparticles (corrosion, dissolution, aggregation…) under relevant natural conditions is crucial for predicting their behavior in ecosystems. Among the various manufactured nanoparticles used in nanotechnology, silver nanoparticles (Ag-Nps) are the most widely used (1) because of their antimicrobial and antifungal properties, and they are being released in significant amounts in natural systems. A major issue, in particular, is the extent to which these nanoparticles release toxic species due to their transformations and dissolution caused by interactions with aqueous solutions.
As type-B metal, Ag react strongly with sulfur. In this context, our study focuses on the sulfidation of Ag-NPs which are among the most used in consumer products as well as the most toxic to a variety of organisms. Our results show that sulfidation strongly reduces the toxicity of Ag-NPs to a variety of organisms. Interestingly, the sulfidated particles are still bioavailable for plants despite their very low solubility (cf. Figure 1). Besides sulfur, Ag strongly reacts with halides such as chloride, which is ubiquitous in natural waters and may strongly affect toxicity. Depending on the Cl/Ag concentration, Cl may catalyze the oxidation and dissolution of Ag-NPs leading to the production of soluble and toxic AgCl species. On the other hand, at low Cl/Ag concentration, Cl may precipitate at the surface of the AgNPs preventing dissolution. Our results show that the toxicity of Ag-NPs on E.Coli can be correlated with the Ag/Cl ratio.
Part of this work is a contribution to the Labex Serenade (n° ANR-11-LABX-0064) funded by the « Investissements d'Avenir» French Government program of the French National Research Agency (ANR) through the A*MIDEX project (n° ANR-11-IDEX-0001-02) and the French X-ray CT platform called Nano-ID was funded by the EQUIPEX project ANR-10-EQPX-39-01.

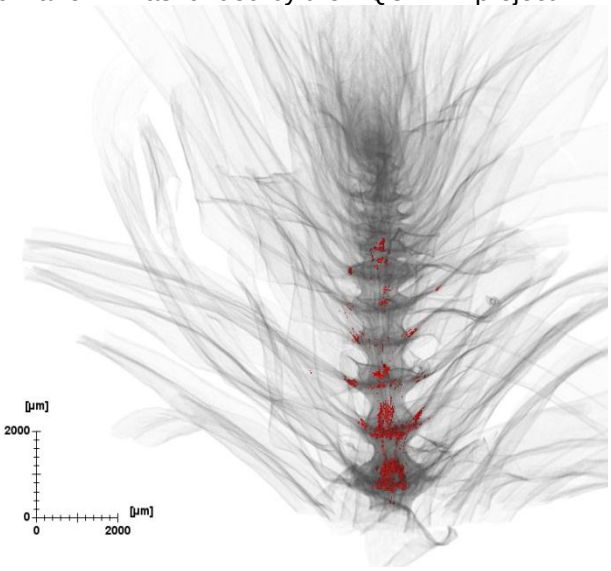

Figure 1. 3D volume of an egeria sample that were exposed with Ag_2S NPs. The 3D volume was collected by micro-Computed Tomography. Red parts represent the denser part of the plant identified as Ag_2S by X-ray Absorption Spectroscopy.

N° O5a-4

COMPARISON OF TIO_2 NANO-OBJECTS TOXICITY ON *CAENORHABDITIS ELEGANS*

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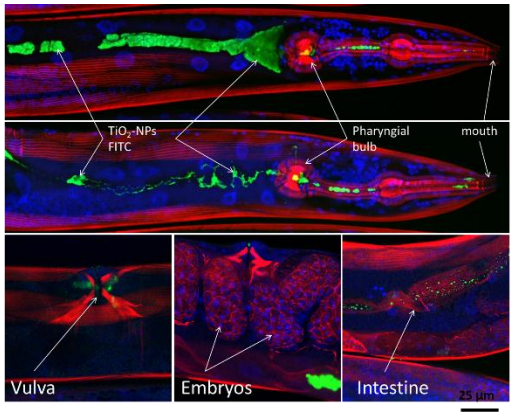
Titanium dioxide nanoparticles (TiO_2-NPs) are mostly used for their electronic properties associated to their low cost and the numerous possibilities in the synthesis and modifications of TiO_2 nanomaterials (1). However, their release and their accumulation in the environment are not well documented. The potential toxicity of TiO_2-NPs on ecosystems and organisms needs to be investigated. *C. elegans* (Invertebrate, Nematode) is a good eco-toxicological model because of its convenient handling in the laboratory and its sensitivity to different stresses (2). In this context, we studied the ecotoxicological effects of three well-characterized TiO_2-NPs various forms (P 25 Evonik, nanotubes, nanoneedles) crystal phases, size, surface area or exposed faces on *C. elegans*.
NPs ingestion was studied by conventional microscopies and ion beam analysis using TiO_2-NPs labelled with fluorophores such as FITC or TRITC. NPs were found in the pharynx and the gut of worms and sometimes in vulva. No translocation in gonad or embryos was observed. First observations by transmission electron microscopy did not show any internalization of either of the three TiO_2-NPs in intestinal or others cells. The toxicity of TiO_2-NPs was investigated using several parameters of the cycle of development of worms: survival, worm length and reproduction. Different exposure durations with several larval stages (L1, adults) were performed in the dark to minimize photocatalytic activation. During exposures, *E. coli* bacteria (food source of *C. elegans*) was not affected by the TiO_2-NPs. The toxicity observed for the different endpoints on *C. elegans* seemed to be depending of TiO_2-NPs characteristics.

(1)Chen, X., Mao, S.S., Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications, and Applications. Chemical Reviews, 2007, 107: 2891-2959.
(2)Leung, M.C.K., Williams, P.L., Benedetto, A., Au, C., Helmcke, K.J., Aschner, M., Meyer, J.N., Caenorhabditis elegans: An Emerging Model in Biomedical and Environmental Toxicology. Toxicological Sciences, 2008. 106: 5-28.



High resolution confocal images of paraformaldehyde-fixed C. elegans.
Fluorescence: Fluorescein TiO_2-NPs (green), phalloidin-Alexa 594 (muscle,red) and Hoescht[33342] (nucleus, blue)
This work is supported by the ANR program "Contaminants, Ecosystèmes, Santé" (TITANIUMS) and the Region Aquitaine (TOXNANO program).

N° O5a-5

NANOPARTICLES INTERACTIONS WITH PLANTS FROM MODEL TO ECOSYSTEM: NANO-DESIGN MATTERS

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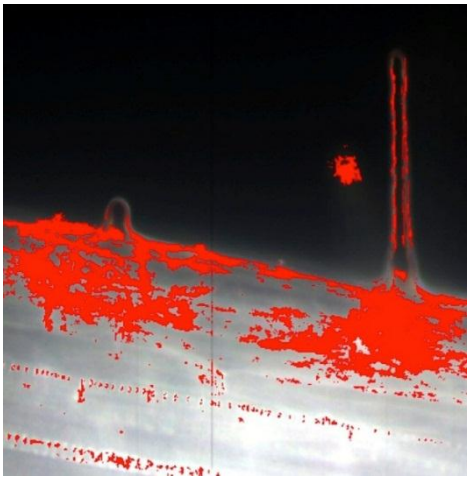
The emerging of nanoparticles industry raises safety concerns about the dissemination of nanomaterials (NMs) in the environment, through uses of nanoenabled consumer products. The primary routes of environmental exposure of plants are through NMs released in air, surface water and biosolids from wastewater treatment plants.
We examined the impacts of cerium oxide nanoparticles on *Arabidopsis thaliana* as a plant model and on a crop plant, *Brassica napus* (rapeseed), *in vitro* and in a soil-based ecosystem. Pristine or citrate-coated nanoCeO_2, with different average particle sizes and surface reactivity were used to assess relationships between nano-design and impacts on the plant at environmental concentrations (1mg/kg).
Interactions of NMs with plants will be addressed by focusing on how NMs modulate plant stress response in acute or long-term exposure to NMs. We will show that CeO_2-based NMs trigger stress response in plants in terms of anti oxidative enzyme activities, plant hormonal pathways and defense metabolites such as phytoalexins. We will focus on how NMs differentially alter plant interactions with their environmental partners such as plant pathogens and soil microbial communities.



Hyperspectral (CytoViva®) Imaging of CeO_2 NMs (in red) interacting with root hairs of Arabidopsis thaliana. Root hairs are important structures that allow plant to get water and nutrients from the soil

N° O5a-6

ENVIRONMENTAL MOBILITY OF CARBON NANOTUBES

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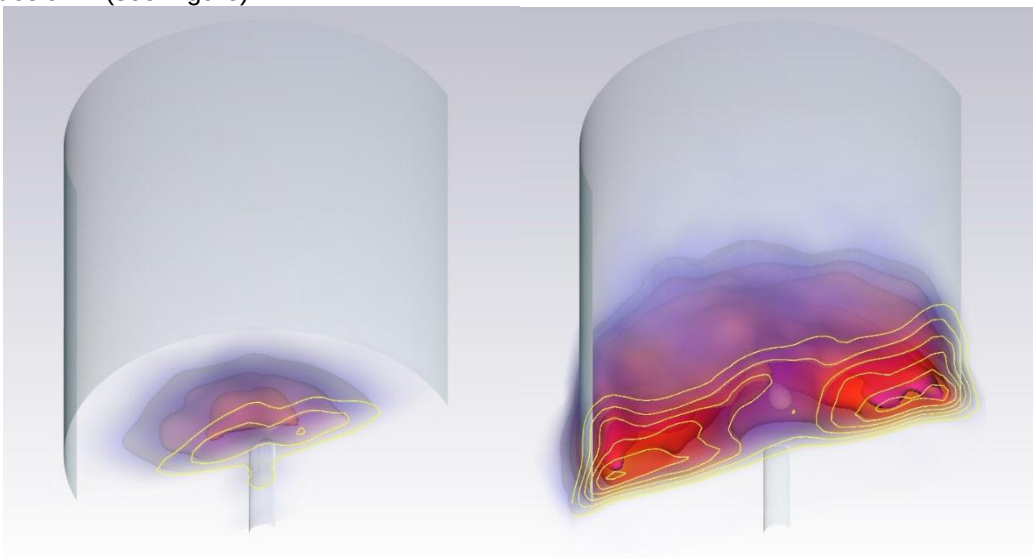
The environmental mobility of nanoparticles is a key factor for the risk assessment of nanoparticle release into the environment. If the environmental conditions render the nanoparticles mobile, a risk beyond the very near field of the actual release has to be taken into account. However, the lack of suitable detection methods constitutes a severe setback for studies of these effects, especially in the low concentration range environmentally relevant and with a considerable background of the same element. This is particularly significant for the study of carbon based nanoparticles such as carbon nanotubes, as carbon-containing water constituents such as humic or fulvic acids are ubiquitous in many ground and surface waters.
We present the results of our studies using carbon nanotubes labelled with radioactive iodine isotopes, e.g. ^{124}I, ^{125}I, ^{131}I. This allowed us to detect carbon nanotubes in the ng/L range, even against a background of mg/L of fulvic or humic acids. Experiments were conducted to investigate the transport behaviour of carbon nanotubes in dependence of the carbon nanotube type, their modification, the geomatrix material and grain size, and the water composition regarding the presence of natural organic matter and electrolytes. The radiolabelling enabled working with a typical amount of about 100 ng carbon nanotubes per experiment. Experiments using different surfactants allowed the distinguishing between different removal mechanisms.
Moreover, the labelling of carbon nanotubes with ^{124}I, a positron emitter, allowed the use of positron emission tomography (PET) to record 4D data (3 spatial dimension plus time) of nanotube transport inside a column (see Figure).



PET image of carbon nanotube transport inside a column, at the beginning of the tracer pulse (left) and after end of tracer pulse with eluent flowing in (right)

N° O5b-1

EVALUATION OF THE EFFECTS OF NITRIC OXIDE-RELEASING NANOPARTICLES ON PLANTS

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Nowadays, there are several commercially available products containing nanostructured materials. Meanwhile, despite the many benefits that can be obtained from nanotechnology, it is still necessary to understand the mechanisms in which nanomaterials interact with the environment, and to obtain information concerning their possible toxic effects. In agriculture, nanotechnology has been used in different applications, such as nanosensors to detect pathogens, nanoparticles as controlled release systems for pesticides, and biofilms to deliver nutrients to plants and to protect food products against degradation. Moreover, plants can be used as models to study the toxicity of nanoparticles. Indeed, phytotoxicity assays are required to identify possible negative effects of nanostructured systems, prior to their implementation in agriculture. Nitric oxide (NO) plays a key role in plant growth and defense, and recently, several papers described the beneficial effects due to application of exogenous NO donors in plants. The tripeptide glutathione (GSH) is an important anti-oxidant molecule and is the precursor of the NO donor, S-nitrosoglutathione (GSNO). In this context, the present work investigates the effects of different concentrations of alginate/chitosan nanoparticles, containing either GSH or GSNO, on the development of two test species (*Zea mays* and *Glycine sp.*). The results showed that the alginate/chitosan nanoparticles present a size average range from 300 to 550 nm with a polydispersity index of 0.35, and encapsulation efficiency between 45 - 56%. The NO release kinetics from the alginate/chitosan nanoparticles containing GSNO showed sustained and controlled NO release over several hours. Plant assays showed that at the concentrations tested (1, 5 and 10 mM of GSH or GSNO), polymeric nanoparticles showed no significant inhibitory effects on the development of the species *Zea mays* and *Glycine sp*., considering the variables shoot height, root length, and dry mass (Figure 1). In conclusion, these nanoparticles seem to have potential for use in agriculture, and might be potencially used as controlled release systems applied using the foliar route.

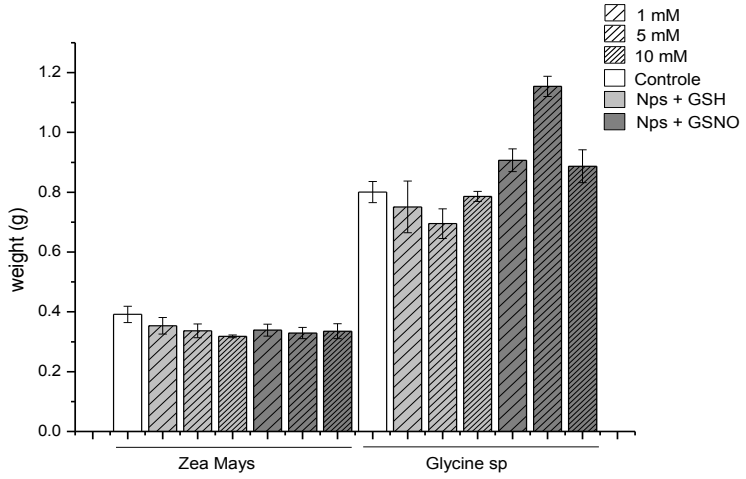

Figure 1. Results obtained for the dry mass of the specimens of Zea mays and Glycine sp. The experiments were performed in triplicate (n=10). The data are expressed as means ± standard deviations; Acknowledgements: Fapesp, CNPq, Capes and Fundunesp.

N° O5b-2

EFFECT OF SILVER NANOPARTICLES ON ESTUARINE BIVALVES *SCROBICULARIA PLANA*

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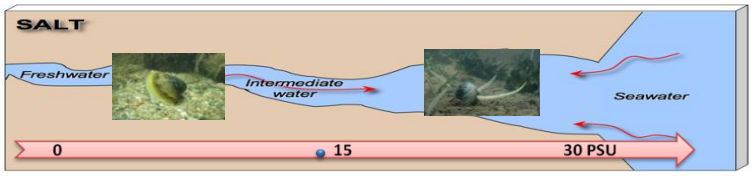
The "nanoSALT" research program is funded by the ANR (French National Research Agency). The aim is to assess exposure and impacts of Engineered Nanomaterials (ENMs) across a salinity gradient taking into account (i) the physicochemical behaviors of Ag and CeO_2 ENMs in exposure media represented by a salinity gradient or salted aquatic media (seawater), (ii) the different stages of the ENMs lifecycle (i.e. formulation, usage, and end of life) and (iii) the ecotoxicity effects towards two bivalve species (*Scrobicularia plana* and *Corbicula fluminea*) covering a large salinity gradient under realistic environmental conditions.

In the present experiment, after acclimatization at two salinities (15 and 30 PSU), *S.plana* has been exposed to low concentrations (10 µg/L) of Ag ENMs: NM-300K (size <20nm, JRC reference nanomaterials) during 7 and 14 days at these two salinities. We also measure the effect of the stabilizing solution of NM-300K.

Ecotoxicity effects have been assessed using a multimarker approach including several biological responses at sub-individual (oxidative stress, metallothionein, genotoxicity, lysosomal stability and cellular alteration) and individual (burrowing rate) levels in the endobenthic- bivalve species.

We observed a significant decrease of the burrowing kinetics of exposed organisms (ENMs, stabilizing agents) compared with controls after 7 days at the low salinity (15PSU). The analysis of the other biomarkers is still in progress. The whole set of biomarkers will be expressed in an integrative way in order to know what and how biological responses could discriminate the effects of ENMs on the bivalve species. We also characterized the dissolution of nanoparticles during the time of exposure (7-14 days). Two different tools were used to study the release of soluble Ag from silver nanoparticles, Diffusive Gradient in Thin films (DGT) and ultrafiltration (Amicon® Ultra-4-centrifugal Filter Devices). Results showed a fast release of soluble Ag from Ag NPs during the time of exposure. Aggregation kinetic of Ag NPs between 15 and 30 PSU was different increasing with the salinity ratio.



Nanomaterials across a salinity gradient: exposure and ecotoxicological effects within a life cycle perspective (production, usage, end of life)

The next step will be to expose both organisms (*S. plana, C. fluminea*) to wound-dressing containing Ag nanoparticles, with feeding and unfeeding system during 7 days. Ecotoxicity effects will be assessed using the multi-biomarker approach indicated above.

N° O5b-3

COMPARATIVE STUDY OF THE TWO TYPES OF NANOPARTICLES ON FRESH WATER MICROCOSM AT LOW LEVEL CONCENTRATIONS

Deepak Kumar[1], N. Chandrasekaran[1], Amitava Mukherjee[1] (1) VIT University, Centre for Nanobiotechnology, Vellore, India, PIN-632014

Nanoparticles are used in different consumer products and industrial applications such as cosmetics and food preservatives etc. The current study was focused to compare the aggregation behaviour of the two different types of nanoparticles i.e. anatase phase of Titanium oxide nanoparticles (TiO_2NPs) and γ–phase of Aluminium oxide nanoparticles (Al_2O_3). The experiments were carried out at low concentration level of NPs (≤1 ppm) to understand the physico–chemical behaviour of the nanoparticles as well as their effect on the microalgae population in a fresh water microcosm. The aggregation behaviour of both types of NPs was studied by evaluating their mean hydrodynamic size for the short period of time (0-12h). The mean hydrodynamic size of TiO_2 NPs increased with increase till it reached micron level whereas the Al_2O_3NPs are stable for 48 hours after that it reached to micron size range. The toxic effect of TiO_2 NPs and the Al_2O_3 NPs was observed for two different time periods i.e short time period (120h) and long time period (180 days). There was decrease in the microalgae population in the microcosm for the short period of time where as in the longer period no such decrease could be noted, which shows that there was no significant toxic effect of the nanoparticles when the exposure period was longer. Thus, the NPs may not have the specific toxic response, at low concentration and longer standing period, probably owing to the complexity of the natural systems.

N° O5b-4

COMPREHENSIVE STUDY ON THE IMPACT OF TIO_2 NPS ON BIOFILM FORMATION OF THE FRESHWATER SEDIMENT BACTERIAL ISOLATES AND THEIR CONSORTIUM: PROJECTED RISKS FOR AQUATIC ENVIRONMENT

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(2) Department of Chemical Engineering, IIT Madras, Chennai, India, PIN-600036

The nanotechnology market is developing rapidly and new applications for nanoparticles are emerging continuously. Titanium dioxide nanoparticles (TiO_2 NPs) are typically used in food, cosmetics, paper, textiles, etc. in a variety of products and the daily exposure to these nanomaterials is reportedly quite high. The transport of stabilized TiO_2 nanoparticles has recently been the topic of extensive research due to its proven potential in causing toxicity in aquatic environment. The current study examines the effects of TiO_2 NPs on the biofilm formation by the freshwater sediment bacteria and their consortium under the UV-light and dark conditions. This is achieved using a representative set up composed of key components of a natural ecosystem. A short term exposure to ecologically significant bacterial isolates (*Bacillus altitudinis*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and their consortium), at environmentally relevant low concentration (≤1 ppm) of TiO_2 NPs, has been studied. The experimental approach was drawn to reveal the physico-chemical behaviour of nanoparticles in a factual aquatic ecosystem. Observational studies showed a significant increase in the biofilm formation and EPS production with an increase in TiO_2 nanoparticles concentration in bacterial isolates and consortium under both UV-light and dark conditions. Additionally the biofilm formation and the EPS production was noted to be more in the TiO_2 NPs treated consortium samples when compared to that by the individual isolates under both light and dark conditions; however, the difference was not statistically significant. Phase contrast microscopy, the SEM provided clear picture of cell aggregation with the help of EPS, membrane damage and morphological distortions. Increase in biofilm production and EPS production due to TiO_2 NPs exposure may favor the survival of the bacteria in adverse environmental conditions. Further studies require to be focused on bacterial surface proteins affected by TiO_2 NPs binding and mechanisms involved in bacterial infection in presence of NPs.

N° O5b-5

AGING OF NANO-PRODUCTS AND IMPACTS TOWARD AQUATIC ORGANISMS ACROSS A SALINITY GRADIENT

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From 2006 to 2010 the number of nano-enabled products (NAP) evolved from approximately 50 to more than 1300. In most of the industrial applications pristine nanoparticles are surface modified and embedded in the final product. It is unlikely that organisms living at the marine continental interface will be in direct contact with pristine nanoparticles. So, there is an urgent need to assess the environmental fate of residues of degradation released from currently commercialized NAPs. To date, several studies have focused on the impacts of NAPs towards freshwater organisms, whereas very few have concerned the impacts towards estuarine and marine organisms.
In this context, we aimed at conducting mesocosms to assess the impacts of NAPs across salinity gradient taking into account the exposure (hetero-aggregation, complexation with natural organic matter, salinity changes). We selected two NAPs based on relevance criteria: fuel additives and dressing containing respectively CeO_2-NPs and Ag^0-NPs. Comprehensive studies of weathering/leaching of NAPs occurring over time as a function of the salinity were performed in environmentally relevant conditions.
Dressing containing Ag^0-NPs were aqueous altered under controlled temperature, pH and salinity conditions. With time, the increasing total concentration of Ag in water highlighted weathering of NAPs and was salinity- and light-dependent. The nano-residues were dissolved Ag and precipitated crystallite of AgCl. So, weathering of dressing leads to a partial dissolution of Ag^0-NPs but not to the release of NPs.
The CeO_2-NPs used in fuel additives were also studied, before and after alteration, by XRD, DLS, IRTF, NMR, XAS and TEM in order to characterize their shape, their size, the nature of the organic coating and the redox state of Ce. Considering life cycle of fuel additives, both aqueous alteration and combustion are conceivable. To mimic combustion underwent by fuel additives in engine motor, we have conducted oxidation with thermo-gravimetric analysis under controlled conditions. Results from these characterizations will give essential information on principles governing NAPs or their residues physico-chemical behavior prior to their ecotoxicity testing across a salinity gradient.

N° O5c-1

GENOTOXIC AND CYTOTOXIC EFFECTS OF SILVER NANOPARTICLES IN THE BIVALVE *SCROBICULARIA PLANA*

Amélie Châtel[1], Pierre-Emmanuel Buffet[1], Hanane Perrein-Ettajani[1], Isabelle Métais[1], Aurore Zalouk-Vergnoux[1], Laurence Poirier[1], Douglas Gilliland[2], Christine Risso-de Faverney[3], Marielle Guibbolini[3],Eva Valsami-Jones[4,5], Catherine Mouneyrac[1]

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Silver nanoparticles (Ag NPs) are widely used in a range of products and processes for their antibacterial properties, electrical and thermal conductivity. Therefore, impact of AgNP release into the aquatic environment is of great concern. Especially since ionic Ag is known to be the most strongly bioaccumulated and toxic element for some marine and estuarine invertebrates and is highly persistent in the environment with a great capacity to accumulate into sediments. In that context, the effects of silver nanoparticles were examined in one of the key estuarine species, the endobenthic bivalve Scrobicularia plana. Experiments were conducted in outdoor mesocosms that closely simulate real-life conditions and allow manipulation of environmental factors. Clams were exposed to 10 µg/L of silver nanoparticles or soluble salt (AgNO3) for 21 days. DNA damage was assessed in both gills and digestive glands of S. plana, using the comet assay. In addition, activities of several enzymes implicated in detoxification mechanisms (catalase, glutathione S transferases (GST), superoxide dismutase (SOD)) and in apoptosis (caspase 3) were measured. Results showed that digestive glands and gills of S. plana depicted a significant increase in DNA strand break levels as well as in activities of catalase, GST, SOD and caspase 3 after exposure to either Ag nanoparticulate or soluble, throughout 7, 14 and 21 days time points, compared to control group. However, higher genotoxic effects of Ag NPs vs soluble Ag were observed after 7 and 21 days of exposure in the digestive glands and after 7 days in gills. Those data suggested a specific effect of silver nanoparticles for some of the conditions tested in this study.

N° O5c-2

CHRONIC CONTAMINATION OF AQUATIC MESOCOSMS BY AG NANOPARTICLES WITH DIFFERENT SHAPE

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The enhanced physicochemical properties of manufactured (NPs) make them highly attractive for a large range of applications. Because of their antimicrobial and antifungal properties, silver nanoparticles (Ag^0-NPs) have numerous uses and are likely to enter in aquatic environments, potentially causing adverse effects not only on humans but also on aquatic ecosystems. Indeed, the oxidative dissolution of Ag^0-NPs in oxic waters produces Ag ions leading to cell death. Other possible mechanisms of toxicity are oxidative stress generated by the formation of reactive oxygen species (ROS) at the surface of the Ag-NPs and “particle specific” effects from Ag-NPs. Unfortunately, most of the toxicity studies published in the literature do not mimic the complexity of natural environments. Many national (e.g. ADEM Afsset and specific ANR) and international programs are devoted to this issue to determine rapidly if these intense uses are feasible. Because of the susceptibility of Ag^0-NPs to transform (changes in aggregation state, oxidation state, precipitation of secondary phases, sorption of (in) organic species), it is important to assess the toxicity of the Ag^0-NPs in environmentally relevant conditions. As an example, Ag^0 has a strong affinity for reduced sulfur (both organic and inorganic) and the sulfidized Ag^0-NPs showed limited acute toxicity compared to non-sulfidized Ag-NPs due to a decrease in their solubility. Chloride can also strongly affect Ag^0-NP solubility and therefore their toxicity. As a consequence, characterization of Ag^0-NP transformation and impact under realistic environment conditions is essential to understand and predict their fate and toxicity in natural aquatic systems.
In this context, to assess the environmental risk of Ag^0-NPs in environmentally relevant conditions we used intermediate size (60 L) indoor aquatic mesocosms to study the distribution and impact of two Ag^0-NPs (plates and spheres) following multiple dosings (chronic contamination). The aquatic environment mimicked was a river ecosystem with gammarus and stoneflies as primary and secondary consumers respectively. Another challenge was to work with NPs concentrations representative of what it is expected in natural aquatic environments (50 µg L-1 of Ag^0-NPs). This study highlighted that the exposure of the organisms and the impacts were not related to the shape of Ag^0-NPs. The impacts toward micro- and macro-organisms will be discussed in term of microbial diversity and oxidative stress.

N° PL6

NANOTECHNOLOGY COMMERCIALIZATION AND THE NEED OF RELEASE TESTING ALONG THE PRODUCT LIFECYCLE

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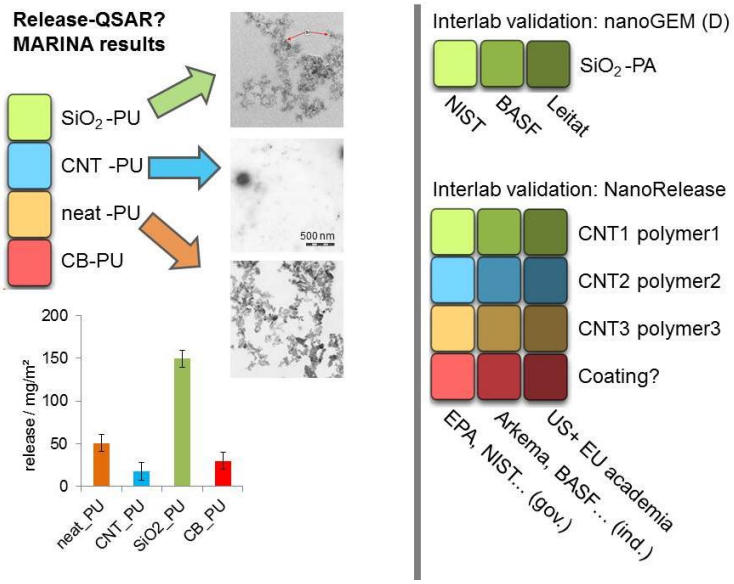
Within material design at BASF we use Nanotechnology to provide sustainable solutions with outstanding performance addressing societal needs. Nanomaterials with new functionalities lead to new products or products with improved performance in electronics, housing and construction, automotive and energy. The diversity of materials and applications is high, ranging from future-oriented products such as energy-efficient organic light-emitting diodes to light-weight composites with embedded nanofillers to monoconstituent polymers with internal structures, such as cm-thick aerogel insulation foams or µm-thin membranes with 20-nm-pores which are used in water treatment. The EC nanodefinition further enlarges the scope towards conventional particulate materials with nanoscale fractions. It is an inherent part of BASF's strategy to apply nanotechnology both with intention and responsibility. Therefore, a comprehensive safety research program and an open stakeholder dialogue aim to support public acceptance. Regulations, if they are reliably established and appropriate for this wide range of materials, can contribute to a transparent and trustworthy development.

One key challenge is to identify those materials that may release nanomaterials at some point of their lifecycle. As „release" case studies, this plenary touches upon how the *MARINA* and *nanoGEM* projects contributed to protocol optimization, thus preparing the ground for the currently ongoing interlab validation in the *NanoRelease* initiative and real-world value-chains in the *SUN* project:

ISO weathering simulation can be extended by sampling and quantification to assess *what* is released from aging nanocom-posites, and at *which rate*. The same applies to drilling and sanding simulations of manufacturing and handling processes.



It is concluded that the release characteristics are highly reproducible between different degradation and sampling protocols. However, the release rates critically depend on aging conditions, so that established ISO standards for aging are beneficial. Systematics of release are emerging: soft matrix vs. brittle matrix, black fillers vs. transparent fillers, particle-shape vs. fiber-shape all modulate the quantitative release rates (bar plot) in a plausible way, but the polydisperse quality of release (see micrograph) is retained under almost all conditions. Benchmarked against the release from aging of pure polymer matrices without embedded nanomaterials, the above consideration of lifecycle releases can be part of a testing and grouping strategy for nanomaterials.

- Release systematics, interlab testing: Carbon (**2014**) 68:33-57; Environ. Chem. (**2014**) 11: 402-418;
- Grouping and phys-chem methods: PFT (**2014**) 11:16; Nature Comm (**2014**) 5:3514
- „Safety of nanomaterial along their lifecycle: Release, Exposure and Human Hazards", Eds. Wohlleben W, Kuhlbusch T, Lehr CM, Schnekenburger J (**2014**) Taylor & Francis, ISBN 978-1-46-656786-3, 430 pages (with contributions from many other NanoSafe participants)

N° O6a-1

CHARACTERIZATION METHODS FOR MWCNT POLYMER COMPOSITES AND NANOCOMPOSITE RELEASE PARTICLES

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As the number of applications and products incorporating multi-walled carbon nanotubes (MWCNTs) increases, the global production capacity for carbon nanotubes continues to grow to match the market demand and so is the concern over the environmental and human health impacts of nano-enabled materials and products throughout their intended and unintended lifecycle stages. Although MWCNTs are used in a wide variety of applications, their use as nano-fillers in polymer nanocomposite materials is one of the most important commercial applications for MWCNTs.
Several recent studies have evaluated and characterized release particles from MWCNT nanocomposites under various simulated use case scenarios [1-3]. Although most studies did not detect free MWCNTs, Schlagenhauf et al. confirmed that free MWCNTs are released from MWCNT epoxy nanocomposites during abrasion experiments [3]. Other studies have indicated possible adverse health effects of MWCNTs even in low exposure scenarios [4-6]. These recent findings combined with the long list of potential applications for which MWCNTs are being considered [7] is raising additional concern for the CNT and CNT product manufacturers as well as regulatory agencies over possible release of MWCNTs, especially free MWCNTs.
One of the biggest challenges in studying environmental and health effects of MWCNTs, and other nanomaterials, is the lack of reliable and reproducible analytical methods and procedures. To that end, we performed a systematic evaluation of MWCNT nanocomposites and their release products using multiple analytical techniques to determine the applicability, usefulness, and efficacy of those measurement methodologies. Epoxy nanocomposite samples with different levels of MWCNT loading were fabricated in-house and the nanocomposite and release particle samples were characterized using the usual suite of imaging and spectroscopic methods such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal Raman imaging. Advantages and limitations of different analytical techniques as well as several types of promising imaging methods are presented.

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N° O6a-2

AN OVERVIEW OF RELEASE FROM SOLID NANOCOMPOSITES

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Conventional composites have been shown to exhibit enhanced durability, strength, or to gain unique properties with the addition of specific nanomaterials. Such nanocomposites have direct consumer and environmental benefits. However, the conditions that induce degradation of these nanocomposites and the composition of the released debris remain poorly characterized. A compilation of the data from over fifty studies designed to specifically investigate release from nanocomposites yield little information in aggregate. Aside from the dearth of experimental studies, most analysis is further limited by the use of disparate methods and materials across these studies. Specifically, the methods used to induce release from nanocomposites have not been rigorously validated. Furthermore, the nanocomposites investigated in many of the studies are novel, lab-made materials with potentially no relevance to commercially viable nanocomposites. And finally, how the released debris correlate to real world conditions remains poorly understood.
What is known at this time is that: (1) most nanocomposites will release particles of matrix alone, and (2) a large subset of these will release matrix particles with the added nanomaterial either partially or fully embedded. (3) Release of the added nanomaterial, completely dissociated from the matrix is only infrequently observed, and (4) ionic forms of the nanomaterial are even more rarely detected. These data highlight that release from nanocomposites can take multiple forms. Importantly, as risk is considered to be a function of the inherent hazards of a substance and the actual potential for exposure, data on nanomaterial release dynamics and debris composition from commercially relevant nanocomposites are a valuable starting point for consideration in fate and transport modeling, exposure assessment, and risk assessment frameworks for nanomaterials. Specifically, method validation and standardization, as well as understanding how laboratory release scenarios relate to real-world conditions would yield beneficial guidance, allowing for more consistent characterization of the release potential of nanomaterials and released debris composition.

N° O6a-3

NANOAEROSOL RELEASE CHARACTERISTICS OF SILVER NANOCOMPOSITE BY SANDING TEST

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For ensuring safety of nanoproducts, nanomaterial release from the nanoproducts should be assessed using repeatable and quantitative methods simulating consumer environment. Environmental aging, mechanical stress, and thermal degradation are considered as potential release scenarios under consumer environment. Abrasion, plastic deformation, sanding, and drilling are typical mechanical processes leading to nanomaterial release. In this work, we investigated nanoaerosol release characteristics from a nanocomposite of silver nanoparticles and polymeric matrix during sanding process using a wear tester. A cylindrical chamber with 100 mm height and 250 mm diameter was installed to isolate released aerosols from ambient air containing large amount of indoor aerosols. To make the chamber clean, particle-free air was supplied into the chamber at a flow rate of 30 L/min. Test were made at three contact loads (10, 30, 50 N) and three rotational speeds (60, 120, 180 rpm) conditions. Each test lasted for 8 min: background check for 1 min, sanding test for 5 min, stabilization check for 2 min. Released aerosols were monitored using a condensation particle counter (CPC, TSI 3010) detecting aerosols larger than 10 nm. During the test, coefficient of friction was also monitored.
As the motor in the tester was turn on, small amount of particles were detected by the CPC. Peak concentrations were less than 30 particles/cm^3 for all test conditions. Note that background particle concentration was less than 0.5 particles/cm^3. Most of particles were released within 30 sec. Sometimes, small peaks were randomly observed during the sanding process. For 50 N contact load, the coefficient of friction was slightly higher than those at 10 and 30 N conditions. The coefficient of friction was insensitive to rotational speed. During the sanding test, total particle number at 50 N was higher than those at 10 and 30 N. On the other hands, total particle number increased with the increase of rotational speed in the range of 60–180 rpm.

N° O6a-4

MEASUREMENT OF NANOPARTICLES RELEASE DURING DRILLING OF POLYMER NANOCOMPOSITES

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Nanomaterials are one of the most promising technologies of this century, and the Project on Emerging Nanotechnologies reports more than 1600 consumer products based on nanotechnology that are currently on the market. In particular, using nano-fillers to reinforce polymeric materials presents many advantages: enhancement of strength, stiffness, scratch resistance, thermal and dimensional stability. Also, only up to 5wt% of nano-fillers is necessary to reach the same improvement observed with at least 20wt% of micro-fillers, which involve cost and weight reduction. However, the concerns about safety and its consumer perception can slow down the acceptance of nanocomposites. During its life-cycle, a nanotechnology-based product can release nano-sized particles exposing workers,consumers and environment and the risk involved in the use and disposal of such particles is not well known. The current legislation concerning chemicals and environment protection doesn't explicitly cover nanomaterials and changes undergone by nanoparticles during the products' life cycle. Also, the possible physio-chemical changes that the nanoparticles may undergo during its life cycle are unknown. Industries need a standard method to evaluate nanoparticles release during products' life cycle in order to improve the knowledge in nanomaterials risk assessment and the legislation, and to inform customers about the safety of nanomaterials and nanoproducts.
NEPHH (Nanomaterials related Environmental Pollution and Health Hazards throughout their life-cycle) project was focused on the identification and quantification of nanoparticles released in the life-cycle of silicon-based polymer nanocomposites. Drilling and crashing experiments were conducted. The results highlighted several issues concerning measurement of airborne particles during drilling tests: particle loss during the measurement, low volume flows of the equipment leading to particle loss and long sampling time, background noises due to uncontrolled ambient environment affecting the results and the importance of controlled drilling parameters (feed rate, rotation speed).
Following these conclusions, SIRENA (SImulation of the RElease of Nanomaterials from consumer products for environmental exposure assessment) project aims to demonstrate and validate a methodology to simulate unintended release of Engineering NanoMaterials from consumer products by replicating different life cycle scenarios to be adopted by the industrial sector in order to get the necessary information for the exposure assessment.
In this work, we will present a new experimental set-up in order to measure airborne particles released during drilling of nanocomposites in a controlled environment to obtain repeatable data set. The custom-made equipment consists of a chamber, a CNC machine, and a SMPS+C for the measurement of the airborne particles. Pre-filter, HEPA filter and a fan are used to provide constant clean air inside the chamber. The CNC machine allows to have control of feed rate and rotation speed of the drill and thus has reproducible and repeatable tests. Also, a water cooled spindle drill is used in order to avoid background noise produced by the drilling motor. Tests have been carried out on three-phase nanocomposites (PA6/Glass Fibre reinforced by nano-SiO_2 or organically-modified Montmorillonite) manufactured by extrusion and injection moulding. The results will be compared with similar tests carried out with the previous system.

N° O6a-5

PARTICLE RELEASE FROM SINGLE-WALL AND MULTIWALL CARBON NANOTUBES IN POLYSTYRENE-BASED COMPOSITES DURING GRINDING

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Carbon nanotubes (CNTs) are an interesting candidate for filler material in composites because of their unique properties. Although composites based on CNTs are expected to be used in a wide range of industrial applications and consumer products, the potential impact of CNTs on human health remain a concern. In order to evaluate the effects and maintain control of CNT exposure, it is important to be aware of potential releases of CNTs throughout their life cycle.
In our previous study [1], we investigated the particle release caused by the grinding of polystyrene-based composites with well-dispersed single-wall CNTs (AIST/TASC Super-Growth CNTs). In the present study, we broadened the range of sample types to include polystyrene-based composites with single-wall and multiwall CNTs (Nanocyl NC7000) that are both well and poorly dispersed.
The experimental method was fundamentally the same as that in the previous study. The composites were ground using a microgrinder in a conductive-antistatic plastic box that had an air-supply opening with a high-efficiency particulate air (HEPA) 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-measuring instruments: a condensation particle counter (CPC), an optical particle counter (OPC), and a scanning mobility particle sizer (SMPS). In addition, the released aerosol particles were collected on Nuclepore membrane filters and holey carbon film-coated grids [2, 3] for analysis using a scanning electron microscope (SEM) and a transmission electron microscope (TEM), respectively.
In the real-time aerosol measurements, increases in the concentration of nanometer- and micrometer-sized aerosol particles were recorded during the grinding of the samples, regardless of sample type. However, similar increases were also observed when CNT-free polystyrene was ground. The nanometer-sized particles were presumably volatile particles released by the frictional heat produced during grinding of the composites [1].
When the aerosol particles collected during the grinding of the CNT-containing polystyrene were analyzed with the electron microscopes, micrometer-sized aerosol particles with exposed CNTs on the surface were observed, regardless of type of the CNT composite. As multiwall CNTs had a larger diameter than single-wall CNTs, the former could be found more easily under the microscopes.

Acknowledgment:

This is based on results obtained from a project commissioned by the New Energy and Industrial Technology Development Organization (NEDO).

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N° O6a-6

TOWARDS HARMONIZED INVESTIGATIONS ON THE RELEASE OF NANOMATERIALS FROM COMPOSITES DURING MECHANICAL TREATMENT – RESULTS FROM AN INTERLABORATORY COMPARISON

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The production and use of engineered nanomaterials (ENMs) has increased at a constant pace over the recent years. At the same time, potential toxic and hazardous effects of these novel materials have raised increasing public attention. Inhalation is seen as the main human uptake route for ENMs and hence the potential release of ENMs during any stage of their lifecycle needs to be assessed. In order for the results to be comparable, harmonized and eventually standardized test procedures are required.

The study presented here is part of the MARINA and nanoGEM projects and focuses on the investigation of possible release of ENMs from composite materials during sanding as an example for a typical mechanical treatment step. Three similar test setups to assess the release of ENM's during sanding have been constructed. In all setups, the material under investigation is mounted to a rotating disc. Sanding paper (grid size 80 or 320) is attached to a stationary stamp and is pressed onto the rotating sample with a defined contact pressure. The mean relative velocity between sample and sand paper is always 0.5 m/s or 1.8 m/s, respectively. The sanding apparatus in each test rig is located inside a sealed enclosure with all other potential particles sources (e. g. electric motors) located outside in order to keep background contamination as low as possible. The number concentration and size distribution of the released airborne dust is characterized in-situ with suitable aerosol measurement instruments. In addition, particle samples are taken for subsequent analyses of the particle morphology and chemical composition.
The three test rigs were subject to an inter-laboratory round robin test. All laboratories received identical samples. These samples were made of either polyurethane (PU) or polyamide (PA) as matrix material filled with different amounts of multi-walled carbon nanotubes (MWCNT), carbon black (CB) or silica (SiO_2), respectively. These samples were then processed under identical settings and test conditions (as far as possible) in all laboratories. The results provide valuable information towards more harmonized studies on the release of nanomaterials. They also reveal that despite largely identical test conditions at all laboratories and test rigs, retrieving comparable data remains challenging. The test rigs and experimental conditions will be presented and the results discussed in view of harmonization of such release studies.

Acknowledgement
The funding of the work presented here by the FP7 Project MARINA (project reference: 263215) and the BMBF Project nanoGEM (project reference: 03X0105A) is gratefully acknowledged

N° O6b-1

NANOMATERIALS RELEASE FROM COMERCIAL FABRICS FOR SPORTSWEAR AND AUTOMOTIVE APPLICATIONS

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Currently the potential impacts of engineered nanomaterials (ENMs) on humans and the environment have generated considerable research interest, since its use and diversity of applications in commercial products have grown extensively over the past decade, and continue to grow rapidly. However, few researchers have addressed the actual release of ENMs, and much more work is needed to evaluate the environmental release and exposure covering its entire life cycle, from ENM synthesis to the end-of-life stage of nano-enabled products.
The main goal of the present work is to study the release of ENMs during the use phase of nano-enabled products, within the framework of the NANOSOLUTIONS FP7 European research project. This project ultimately aims at identifying and elaborating those characteristics of ENMs that determine their biological hazard potential by providing a means to develop a safety classification of ENMs. Two different commercial fabrics have been chosen, in collaboration with the industry: a) sportswear textiles containing Ag and TiO_2 nanoparticles, and b) a polyurethane-based coating on a knitted fabric, with embedded multi-wall carbon nanotutes (MWCNT). It is well known from the literature that Ag and TiO_2 are most frequently added to textiles for antimicrobial activity or UV protection, respectively, while MWCNT can provide anti-static properties.
In order to mimic a real use scenario, a simulation reproducing the washing in normal household conditions will be carried out for the sportswear textiles. Experiments will be performed in a lab-washing machine, based on the international standard ISO 105-C06:1994 for determining color fastness in commercial and industrial laundering. The same process of textile parts rubbing against each other takes place as during washing, and this process presumably is responsible for the release of particles and fibres from a textile to the water compartment. The morphology and size of the textile fibers will be evaluated by SEM. Individual / aggregated released nanoparticles will be identified and its chemical composition will be defined by TEM-EDX. The concentration of Ag and Ti in collected waters will be determined directly by ICP-MS.
On the other hand, a Martindale Abrasion Tester will be used to test the durability of the anti-static cover containing MWCNT, examining the release of particles due to the abrasion process. With this purpose, the standardized protocol ISO 5470-2:2003 will be followed. The nanocomposite will be characterized by means of TEM, FT-IR and TGA, before and after the experimental test. Likewise, during the simulation, released nanomaterials will be directly collected in TEM grids by using a nanoparticles collector and further characterized by electron microscopy.
On the basis of the results of these experiments, we will obtain a more realistic environmental release estimates from the use of textiles containing nanomaterials.

N° O6b-2

TRACKING NANOMATERIALS THROUGH THE LAUNDRY WASH CYCLE: RELEASE, DISSOLUTION AND COMPLEXATION

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In the context of assessing the potential risks of nanomaterials, a lifecycle approach (covering production, use and disposal of the nanomaterial or nano-product) represents a holistic view of their potential impacts. Considering nanomaterial life cycles are determined by application within a product, relevant exposure scenarios and particle aging/transformations are strongly dependent on the life-cycle of nano-enhanced products themselves. For example, nanomaterials embedded in textiles would transform after exposure to oxidants and detergents during washing. Building on previous results which demonstrated the quantity and general size fractions of various silver additives released from textiles, we further characterized specific nanoparticle properties in the laundry wash cycle. Using a number of different liquid and powdered commercial detergents, simulated washing studies were preformed on nanoparticles suspended in wash solution and released from textiles to better understand particle release, (change to) particle size distribution, dissolution, re-precipitation (i.e. « new » particle formation) and complexation of manufactured nanomaterials to particulate matter in the washing solutions, such as zeolite. Ag NPs were studied for their relevance to the textile industry alongside Ag^+ (added as $AgNO_3$) which served as a baseline for any particle re-precipitation or Ag^+ adhering to the particulate matter in the wash solution. Au NPs were used as a « tracer » through the system, since it is less reactive or prone to dissolution, to ensure analytical characterization techniques did not distort particle size or number concentrations throughout the tests. TEM and EDX analysis were performed to observe morphological and chemical changes to the particles after washing. Single particle ICP-MS (spICP-MS), capable of analyzing the size of individual particles in the ng/L concentration range, was used to build a size distribution of particles in solution and detect complexation with larger floc material in the washing solution. Furthermore, unique advancements to the spICP-MS method were implemented here to track individual particles and determine if the original nanomaterials were present in the wash solution or, rather, if particles that were measured after the wash cycle were « new » particles which had precipitated from dissolved silver. These studies help to further the understanding of nanomaterials through the product life cycle and give a better indication of how nanomaterials will be aged and transformed over time during regular nano-enhanced product use.

N° O6b-3

EFFECTS OF RELEASED PARTICLES: NANOHOUSE RESULTS

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Introduction

The use of nanoparticles has led to the development of paints with improved antimicrobial properties, durability, fire resistance and self-cleaning characteristics. Due to these beneficial effects, the paint and coating industry has become an important users of nanoparticles. Because nanoparticles possess novel properties, biokinetics and unusual bioactivity, their biological effects are likely to be different compared to larger (non-nanoscale) particles.

Objectives

The toxic effects of nanoparticles embedded in a paint matrix compared to pristine nanoparticles.

Results

We studied the toxic effects of pristine nanoparticles (TiO_2, Ag and SiO_2) and aged paints with and without nanoparticles both *in vitro* as *in vivo*. Two of the three pristine nanoparticles (TiO_2 and Ag) showed some cytotoxic effects at relative high doses using a biculture consisting of human bronchial epithelial cells and human monocytic cells, while all aged paints with nanoparticles and pristine SiO_2 nanoparticles did not. After pulmonary exposure of mice to the different particles, the pristine Ag nanoparticles showed the most pronounced response (increase in neutrophils, pro-inflammatory cytokines KC and IL-1ß), while the other pristine nanoparticles (TiO_2 and SiO_2) showed only a subtle toxic effect in the lungs. The paints containing nanoparticles did not show significant toxicity. Our results suggest that addition of nanoparticles (TiO_2, Ag and SiO_2) to paints and coatings do not pose an additional hazard for human health.

Conclusion

The results help to fill some knowledge gaps and form an added value to the existing literature in the field of nanotoxicology. We hope this work is a step towards the safe design and development of new materials and consumer goods containing nanotechnology in the future.

Acknowledgements: This work is part of the EU FP7 project: NanoHouse (ID 207816*)*

N° O6b-4

LEACHING POTENTIAL OF NANOMATERIALS DURING DIFFERENT HUMAN CONTACT SCENARIOS AND END-OF-LIFE

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In order to understand how much, when and by which mechanisms nanomaterials are released during the life cycle of a given application, we have experimentally investigated the release of nanoparticles (NP) from a wide range of products. These include silver and titanium dioxide NP released from food storage containers, titanium dioxide released from coated ceramic tiles, iron (III) oxide NP from polyethylene granulates and silver NP released from toothbrushes. In our investigation, we focused specifically on release during the consumer use phase and the waste handling phase as these two aspects of the life cycle seem to be especially important and not well understood. In order to get an estimation of the overall release potential of nanomaterials during the consumer use phase and the waste phase, we also mapped consumer products on the EU marked claiming to be nano-enabled and commercially available online (see www.nanodb.dk) as well as the waste flows of these consumer products. We identified more than 1275 products to be available in the EU. Almost 200 products of these are claimed to contain nanosilver, but for more than 800 products the identity of the nanomaterial used was not reported. Based on information available online, the consumer products were categorized into waste material fractions, and we found that “Dirty plastic” (e.g. used bottles and containers) was clearly the dominating waste fraction for nano-enabled products. CNTs and other nanomaterials were primarily represented in one or two waste fractions, whereas nanosilver was found to be present in six of the eight identified waste fractions.

N° O6b-5

WHAT IS EMITTED FROM COMBUSTION OF NANOCOMPOSITES? RESULTS ON PU AND PE POLYMERS WITH CARBON BLACK, NANOTUBES, IRON OXIDES, ORGANIC PIGMENTS.

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Nanotechnology is one of the strongest innovation drivers in research and development today. Within material design at BASF we use Nanotechnology to provide sustainable solutions with outstanding performance addressing societal needs. But the proliferation of Nano-enabled-Products (NEPs) has inevitably raised the urgent question of nano-release during their synthesis, integration, processing, assembly, usage and eventually recycling or disposal at the end of their life cycle.
Here, we use the recent development of a novel exposure generation platform to study and understand in a systematic manner the release mechanisms of NEPs during incineration. The result shown in Figure 1 for the combustion of polyethylene (PE) containing Fe_2O_3 (32nm) is exemplary for all experiments: release into aerosol was rarely observed, but nanofillers remain in the ash, even a fraction of those that are combustible. The identification by IR, OC/EC ratios and TEM / EDX is complemented by the detailed and time-resolved characterization of the emitted aerosols in terms of size distribution and concentration, throughout the combustion process. The combustion process depends on the nature of the polymer matrix (PE vs. polyurethane (PU)), with only minor influence by the embedded nanomaterials.

AEROSOL	**RESIDUAL ASH**	
TEM 0.1–2.5µm fraction	SEM scan	EDX of SEM scan

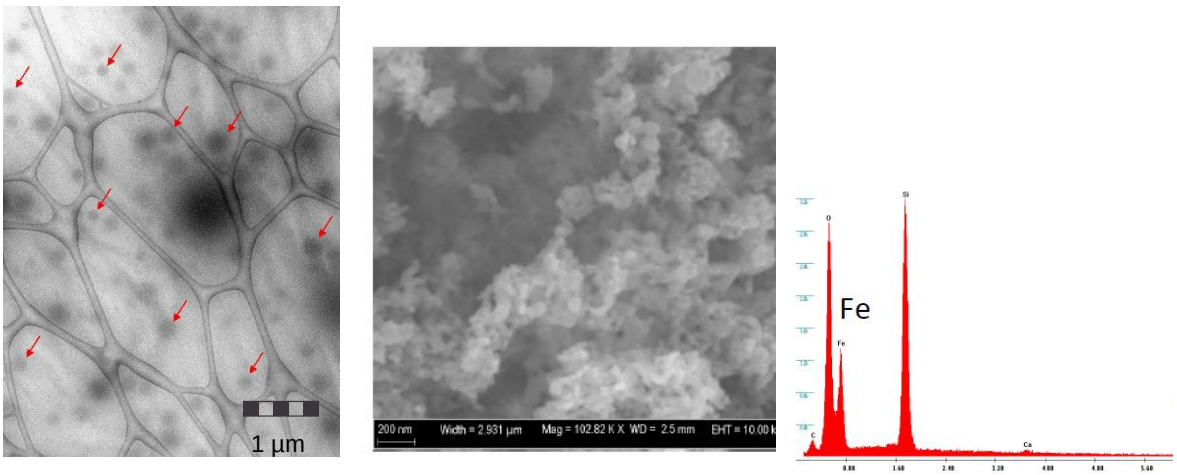

Figure 1: Combustion of PE containing Fe_2O_3 at 500°C, O_2 reduced by 0.2%. While the aerosol is dominated by volatile organics, identified by IR, TEM and denuder manipulation, the nanoscale iron oxide remains in the residueal ash, identified by SEM and EDX.

N° O6b-6

CHARACTERISING THE RELEASE OF CARBON NANOTUBES FROM BURNING CNT-POLYMER NANOCOMPOSITE

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Carbon nanotubes (CNTs) exhibit unique properties that have attracted considerable interest from industries for novel applications. One such application is the incorporation of CNTs as fire retardant additives in polymer matrices to be used as safer alternatives to brominated and chlorinated flame retardants in commodity plastics. They have shown the potential to reduce heat release rate and increase time to ignition in a number of polymers [1, 2]. However, *in vivo* and *in vitro* studies have shown that inhalation of free standing (long and straight) CNTs can cause inflammation, immune response and in some cases fibrogenesis, cancer and translocation to extra pulmonary organs [3, 4]. There is potential for human exposure through manufacturing processes, use, end-of-life; and when CNT-polymer nanocomposites are exposed to accidental or deliberate fires. Mechanical forces applied to CNT-polymer nanocomposites show minimal release of free standing, respirable CNTs in the atmosphere [5]. Nevertheless, few investigations exist to date that assess the potential release and behaviour of CNTs, embedded in polymers, from different fire scenarios [6]. This is due to the difficulty of replicating real fire conditions, but also identifying, detecting and distinguishing these nanoparticles in the complex carbonaceous residues and airborne emissions from fires.
In this study, the possibility of CNT release from burning CNT-polymer nanocomposite pellets (Polyamide 6.6 containing: 0%, 1% and 2% CNTs) under different fire conditions by using several qualitative microscopic and spectroscopic techniques is investigated. The polymers were combusted by using the pyrolysis/combustion tube furnace NF X-70-100, at temperatures ranging from 400-800°C. Samples from the residual soot and gas phase emissions were analysed by electron microscopy and Raman microscopy.
This work is funded from the European Union's Seventh Framework Programme FP7/2007-2013 under grant agreement n°308391.

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N° O6c-1

DEVELOPMENT OF A CONCEPTUAL FRAMEWORK FOR EVALUATION OF NANOMATERIALS RELEASE FROM NANOCOMPOSITES: ENVIRONMENTAL AND TOXICOLOGICAL IMPLICATIONS

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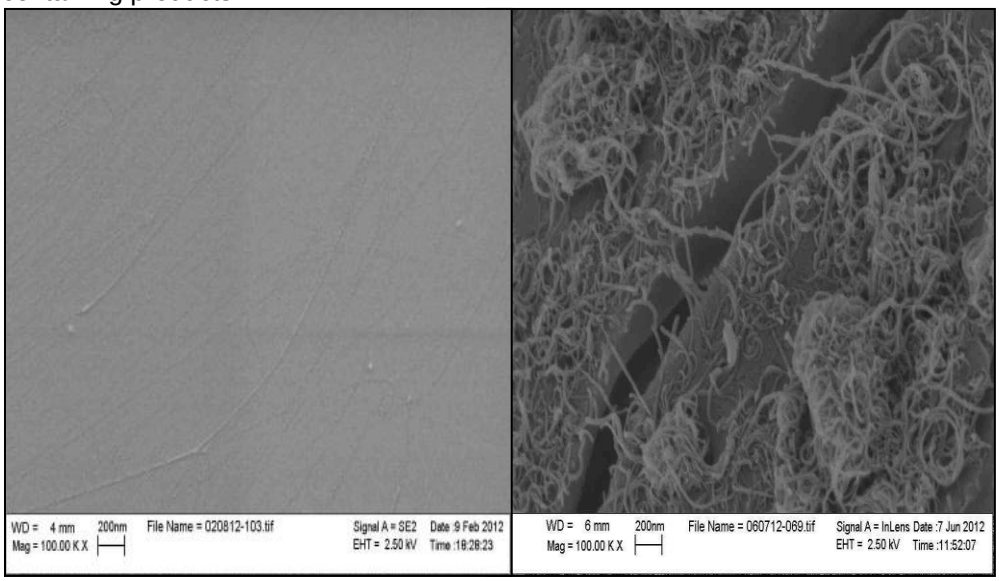
Despite the fact that nanomaterials are considered potentially hazardous in a freely dispersed form, they are often considered safe when encapsulated into a polymer matrix. However, systematic research to confirm the abovementioned paradigm is lacking. Our data indicates that there are possible mechanisms of nanomaterial release from nanocomposites due to exposure to environmental conditions, especially UV radiation. The degradation of the polymer matrix and potential release of nanomaterials depend on the nature of the nanofillers and the polymer matrix, as well as on the nature of environmental exposure, such as the combination of UV, moisture, mechanical stress and other factors. To the best of our knowledge there is no systematic study that addresses all these effects. We present here an initial study of the stability of nanocomposites exposed to environmental conditions, where carbon nanotube CNT-containing polymer composites were evaluated with various spectroscopic and microscopic techniques. This work discusses various degradation mechanisms of CNT polymer nanocomposites, including such factors as UV, moisture and mechanical damage. An in vivo ingestion study with Drosophila showed reduced survivorship at each dose tested with free amine-functionalized CNTs, while there was no toxicity when these CNTs were embedded in epoxy. In addition to developing new paradigms in terms of safety of nanocomposites, the outcomes of this research can lead to recommendations on safer design strategies for the next generation of CNT-containing products.



Degradation of CNT-containing composites. (Left) surface of CNT-Epoxy surface before exposure to UV; (Right) magnified agglomerate of nanotubes on the surface of sample exposed to UV for 1308 h, showing cracking of the matrix and partially unprotected nanomaterials.

N° O6c-2

MECHANISMS OF ENTANGLED CNT LAYER FORMATION AND ITS RESISTANCE TO RELEASE DURING UV IRRADIATION OF POLYMER NANOCOMPOSITES.

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Multi-walled carbon nanotubes (MWCNTs) have excellent mechanical, electrical, and thermal properties. This material is being investigated extensively as a nanofiller to enhance multiple properties of polymeric materials. Under UV radiation, however, the polymer matrix is degraded, and MWCNTs are observed to accumulate on the nanocomposite surface. This talk will first present our research results and from the literature to show that the CNT layer formed on the nancomposite surface due to UV irradiation is a complex, entangled network that, in the absence of strong external forces, does not release individual MWCNTs to the environment. The second part of the talk will present possible mechanism for the formation of the entangled MWCNT layer on the nanocomposite surface caused by UV radiation and the mechanism for the strong resistance of this entangled MWCNT layer to release during weathering.

N° O6c-3

LIFECYCLE OF COMMERCIAL PHOTOCATALYTIC NANOCOATINGS: NANOPARTICLES AEROSOL EMISSION DURING MECHANICAL AND ENVIRONMENTAL STRESSES APPLICATION

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The application of the photocatalytic nanocoatings on external walls of buildings is ever increasing due to their anti-bacterial and self cleaning properties. Such properties are ensured by the photocatalytic action of titanium dioxide (TiO_2) nanoparticles. Resting on the external surfaces, these nanocoatings are frequently subjected to various mechanical solicitations and environmental weathering in real life conditions [1]. As a result, the consequent loss in their structural integrity leads to their disintegration which, in turn, may lead to the exposure of embedded nanoparticles and thus their possible release too. For a durable development, understanding their ecological and human health effects is important. In the last decade, slowly though, this concern has started gaining attention. Various studies have demonstrated toxic effects of TiO_2 nanoparticles.

In the present work, a parametric study on the emission of TiO_2 nanoparticles from two commercial photocatalytic nanocoatings is carried out [2]. For this, abrasion tests are performed on them [3] inside a background particles free work post [4]. The effects of varying contact pressure during abrasion [5] and number of layers of the nanocoating on the number concentration, size distribution, shape and chemical composition of the generated aerosol particles are studied. To study the effect of the accelerated weathering on the nanoparticles emission into air and water, one of these two nanocoatings is chosen and exposed to UV rays and humidity from 1 to 7 months under standardized conditions [6].

The two nanocoatings appear to exhibit contrastingly opposite behavior in terms of the aerosol particles emission inhibition. Irrespective of the type of nanocoating and weathering, the aerosol particles always possess irregular shapes with unimodal size distributions. In terms of Ti content, while the non-weathered nanocoating samples generate aerosol particles with 1.5–3.5% (in mass) of Ti content, the aerosol generated from weathered nanocoating samples have dramatically increased Ti content (~52%) by the end of 6 months of weathering. By 7 months, a considerable presence of free TiO_2 nanoparticles starts to appear too. The presence of TiO_2 in the leachate water samples is not detected under present experimental conditions.

Three indicators - *Emission Transition Pace* (ETP), *Stable Emission Level* (SEL) and *Stable Emission Duration* (SED) are evaluated and found to be pertinent to qualitatively assess a nanocoating's useful life and monitor the particles emission from it.

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N° O6c-4

IN SEARCH OF FACTORS AFFECTING THE RELEASE OF NANOMATERIAL FROM PRODUCT'S LIFE CYCLE: THE GUIDENANO PROJECT

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The evaluation of chemicals and materials release into the environment is a starting point for the assessment of environmental exposure. In terms of nano-enabled products where nanomaterials are embedded in or coated onto a matrix, the release of nanomaterials would be an initial step in most exposure scenarios. Exposure can occur at many points in the life cycle of nano-enabled product. There is a potential for occupational exposures during e.g. synthesis of NM; exposure to consumers while the nano-enabled product is being used (e.g. nano-enabled textile); and exposure to both the general population and the environment when release occurs during the use phase or product disposal. Although the release assessment is a prerequisite to exposure studying, very little attention has focused on understanding the conditions for release of NM from product's life cycle.
Within the EU-funded GUIDEnano project, we identify approximately 80 studies that specifically investigated the release of nanomaterials, and we reviewed them in order to identify nano-enabled product tested (composition, size, product category, etc.), the life cycle phase investigated, the experiment details (pH, temperature, leaching conditions), the environmental compartment affected (air, water, soil), the features of released NM (amount, aggregation, shape) and the main factors driving the release (UV exposure or mechanical stress). These data were then organized in a release library, which is related to all life cycle stage of nano-enabled product (NM synthesis, manufacturing of nano-enabled product, use phase and end of life phase of product). Although differences among the experimental design make comparison difficult, the release of nanomaterials in the environment depends on several factors, which were categorized as follow: (1) intrinsic physical-chemical properties of the nanomaterial; (2) interaction nanomaterial-product matrix; (3) environmental (weathering) conditions; (4) process and manufacturing activities.
Although the analysis of complex systems with significant uncertainty as the estimation of nanomaterial release from the whole life cycle of nano-enabled, our analysis allowed to define first decision tree for estimating the nanomaterial release for both use phase and end of life. These decision trees (fig. 1) containing release factors will be further elaborated in the future in order to include them within the GUIDEnano Tool.

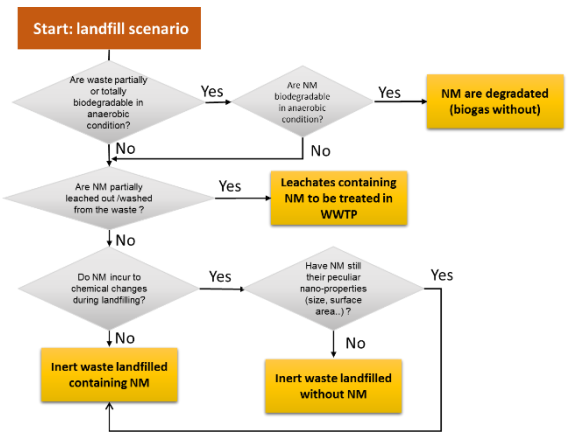

Figure 1. Preliminary decision tree developed to estimate potential release of nanomaterial from disposal of nano-enabled product.

N° O6c-5

INSIGHT INTO MECHANISMS LEADING TO THE RELEASE OF CeO_2 NANOPARTICLES EMBEDDED IN AN ACRYLIC WOOD COATING

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The study of nanomaterials release from solid matrices is an emerging field of research. Until now most efforts have focused on quantifying and identifying the released objects, providing valuable inputs to risk assessment models. However the mechanisms lying behind release are still largely unknown and rarely investigated.
Understanding release mechanisms of nano-objects is critical under two aspects. First of all it may allow predicting NOAA (Nano-objects, their aggregates and agglomerates) release based on a few material properties and may then reduce the need for costly and time-consuming testing. In a second time, unveiling release mechanism is key to implement a safe-by-design approach of nanotechnology. Once elucidated, physico-chemical processes leading to nanomaterial leaching can indeed be counterbalanced, to gain a better control on NOAA emissions.
Nanomaterials have wide applications in paint and coating industry. They can improve rheological and mechanical properties of the products, confer them self-cleaning or antimicrobial capacity, or act as UV-absorber, stabilizing agents, pigments, etc. Along their life cycle paint and coatings will however experience processes that may lead to NOAA release. This is especially true for outdoor products, as sunlight and rain can induce very strong degradations.
A weathering protocol in climatic chamber was developed at CEREGE, to evaluate NOAA releases from coatings under laboratory conditions. Alternating irradiation under Xe lamp ($60W.m^{-2}$ in the 300nm-400nm range) and "rain" (=water spraying) phases were applied to an acrylic protective wood coating, enriched with CeO_2 nanoparticles. Over a 3-months assay, significant emissions (> $1mg.m^{-2}$) of particulate CeO_2 into water could be evidenced.
A thorough characterization of wood samples was performed in order to understand the mechanisms leading to CeO_2 release. Optical microscopy revealed the presence of cracks and blistering on weathered samples. It also showed an increase in paint porosity. In parallel, infrared spectroscopy and nuclear magnetic resonance were used to analyse chemical degradation of the acrylic polymer matrix.
The overall distribution of CeO_2 nanoparticles in the coating was assessed from results of X-ray fluorescence microscopy and laser-ablation-ICP-MS. Complementarily, direct size measurements on CeO_2 aggregates incorporated in the wood coating were performed by micro and nano X-ray computed-tomography. They proved that aggregation of nanomaterials took place upon aging. Further transformation of ceria nanoparticles within the coating was evidenced by XANES, which showed partial reduction Ce(IV) to Ce(III) along the experiment. Based on this data, hypothesis on both the processes lying behind release and the form under which CeO_2 is released is presented.

N° O6c-6

INVESTIGATION OF THE NANOPARTICLES RELEASE MECHANISM FROM PAINTS DUE TO ENVIRONMENTAL AND MECHANICAL AGING

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Nanoparticles, which by convention mean objects having at least one dimension within the nanometer range (1-100 nm), are daily used in new industrial applications. In the field of paints, the addition of nanoparticles modifies their formulations. In function of their use, different nanoparticles are incorporated in paints to improve their mechanical properties and their UV resistance but also to control their rheological properties and their coloring for example. This emerging industry takes into consideration a responsible development of their products towards humans and the environment (eco-design). This requires in particular the security control of the nanoparticles added in consumer products throughout their entire life cycle: manufacturing, use and end of life phases. As no reliable toxicity data for all nanoparticles are known today, current studies focus on the “non-release” phenomenon of the nanomaterials.
The presented project is based on the characterization of nanoparticles released from paints; it follows results obtained within the frame of the NanoHouse project. A wider range of paints formulation was studied and several types of solicitations of use were tested (mechanical and environmental). The aim of this project is to better understand parameters that control the release of nanoparticles and to improve the paints formulation to prevent this phenomenon. Thanks to these results, we expect to provide the necessary elements to establish safe-by-design paints for their introduction on the market.
In our paints, nanoparticles were added to the formulation as pigments: TiO_2, SiO_2, Carbon Black and others organic pigments. Firstly, they were characterized to determine their chemical natures, particle size distributions and particle shapes with Scanning Electron Microscopy coupled with Energy Dispersive Spectrometry (SEM-EDS), Cryogenic Transmission Electron Microscopy (Cryo-TEM) and Dynamic Light Scattering (DLS). In order to simulate outdoor aging during the life cycle of the product, painted panels were exposed to accelerated weathering experiments respecting the norms EN ISO 16474-3. Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, X-Ray Photoelectron Spectrometry (XPS), Contact Angle Measurements with Sessile Drops and SEM-EDS were used to investigate surface modifications of theses paints. These experiments allowed us to analyze the surface of each samples and to understand the weathering effect on paints, its impact on nanoparticles release and on the interface between polymer and pigments. After this previous aging, abrasion tests were performed with a Taber Abraser to simulate the mechanical aging. The particle size distributions and the quantification of aerosolized abraded particles were performed with an Electrical Low Pressure Impactor (ELPI), with a Fast Mobility Particle Sizer (FMPS) and with a Condensation Particle Counter (CPC). With the observation of the ELPI collecting filters by SEM, morphologies of the aerosolized particles (free or agglomerated nanoparticles or embedded nanoparticles in polymer matrix) were determined, allowing to conclude on their potential danger.

N° PL7

NANOMATERIALS: INDUSTRIAL PRODUCTION AND PREVENTION

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Nanotechnology is a field of research and industrial development that focuses on structures, devices and processes based on the atomic, molecular or supramolecular modelling of matter at scales typically of the order of one to one-hundred nanometers (1 – 100 nm).
The constituents—sometimes referred to as elementary bricks - are nanomaterials that are only produced in small quantities. Nanomaterials include nanoparticles, nanostructured coatings, dense bulk materials and nanocomposites (with an organic, inorganic or metal matrix).
This paper will focus on industrial productions, the current practices to prevent nanorisks and the new ways of prevention as the safer by design / process based approaches.

N° O7a-1

DUSTINESS TESTING: A SUPPORT TO NANOSAFETY BY DESIGN

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Nanotechnology is often said to be the industry of the 21st century. Its growth and development may pose potential occupational health and safety issues. The "safety-by-design" approach may be a suitable solution to address these issues. It consists in taking into account the risks (exposure and hazards) and performances in the manufacturing of the future nanomaterial-based products to enhance their safety over their entire lifecycle. Synthesizing nanopowders is one of the techniques to elaborate nanoproducts. One of the major risks related to the powders is that of aerosolization. Indeed, particle release at the workplace (inhalation risk) is not to be underestimated and is therefore to be taken into account in the nanosafety-by-design approach. As a consequence, there is a need to develop experimental systems and protocols to assess dustiness of the powders.
Among all the known techniques available to assess dustiness, the vortex shaker appears to be most promising for nano-dustiness [1, 2]. This system is based on the use of a "vortex" type mixer onto which a glass test tube containing the sample is mounted. The test tube is sealed with a stopper pierced with quarter-inch diameter stainless steel inlet and outlet tubes. Clean air is flowed in the glass test tube through the inlet. Air circulation within the glass tube combined with shaking makes the aerosolized fraction of the powder flow out through the outlet tube.
Dustiness experiments have been carried out on ZrO_2 powders produced by the PLASMACHEM company in the frame of the SANOWORK project (7th European Framework Program, GA: 280716). The studied materials included one original (unmodified) and three "modified-through-design" powders. The modified ZrO_2 samples were synthesized with the aim to reduce aerosolization. The objective of these experiments was therefore to compare the dustiness of the original and modified powders.
Several tests were performed on the powders and their aerosolized fractions. First, SEM observations were made to visualize the powders. The temporal evolution of the number concentration of the aerosolized fraction of the powder was monitored using particle counters whereas the aerosol composition and morphology was characterized by TEM analysis.
The experimental results show significant reduction in the dustiness of some modified powders. These results make it clear that efforts made to modify powders in order to make them less emissive are worth implementing to reduce the inhalation risks. They also highlight dustiness tests as a simple and efficient tool to assist remediation strategies.

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N° O7a-2

WET STATE CHARACTERIZATION AS KEY STEP IN A SAFETY BY DESIGN APPROACH

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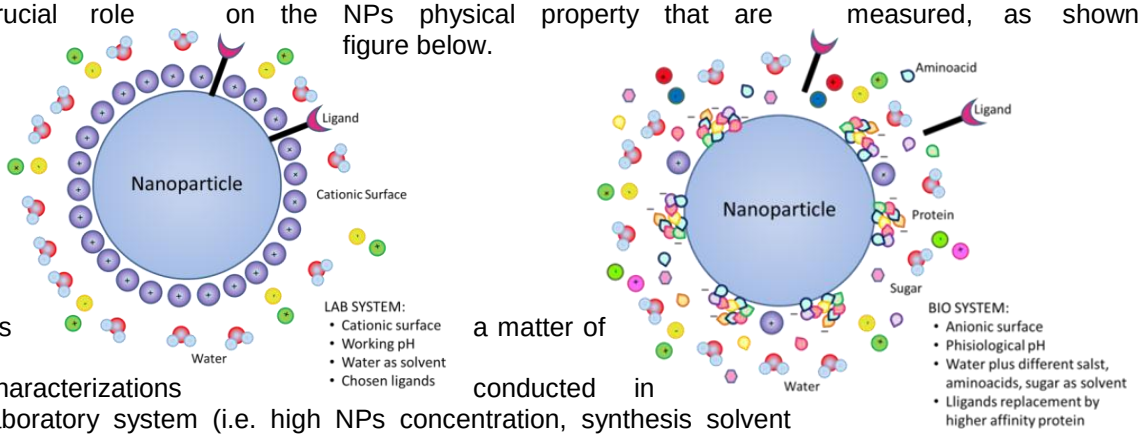
In the frame of collaborative European project SANOWORK (FP7-NMP-2011-SMALL-5) different surface modification have been applied to make nanoparticles (NPs) "safest by design". To propose a safe and efficient application of NPs, is of prime importance to characterize them in the "environment" where they will move and react. Wet state characterizations are fundamental to predict NPs identity and reactivity toward biological matter, especially if simulate specific biological conditions, such as low NPs concentration, physiological pH, presence of protein, amino acids and salts, that could play a crucial role on the NPs physical property that are measured, as shown in figure below.



As a matter of fact characterizations conducted in the laboratory system (i.e. high NPs concentration, synthesis solvent and pH) over simplify the conditions that NPs encounter in bio-system, with a consequent wrong information collection, that hinders the definition of sound structure-reactivity mechanisms and limit the success of safe by design approach.
In this contest a set of samples from SANOWORK project, samples (TiO_2, pristine and surface modified on the basis of "Safety by design" concepts), have been characterized both in lab- and bio-system. Biological conditions are imitated using time, temperature, concentration and solvent t closely simulating the experimental conditions used for cellular tests. The interaction of NPs with serum protein was investigated both using bovine serum albumin in phosphate buffered saline solution, as model system, and under in vivo cell culture condition (complete culture media).
Different characterization techniques like as zeta potential and size measurement, UV-vis spectroscopy, transmission electron microscope (TEM), surface enhanced Raman scattering (SERS) and attenuated total reflectance infra-red spectroscopy (ATR-IR) together with less conventional techniques as nuclear magnetic resonance (NMR) and X-ray photoelectron spectroscopy (XPS), were employed to monitor the NPs surface changes and to explore the "nano-bio" interactions.
Data from a single characterization technique are sometimes not readily amenable to interpretation and needs careful consideration, but merging the information given by different tecqniques, more sound results and conclusions can be achieved. The characteristic of NPs both in laboratory and biological systems are compared and the latter one have been correlate to in vitro test to deep understand the nano-bio interactions with the possibilities to improve the design of safest nanomaterials.
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N° O7a-3

SAFETY BY DESIGN TO CONTROL THE BIOLOGICAL REACTIVITY OF NANOSILVER

Magda Blosi[1], Simona Ortelli[1], Camilla Delpivo[1], Davide Gardini[1], Massimiliano G. Bianchi[2], Manfredi Allegri[3], Ovidio Bussolati[3], Enrico Bergamaschi[2], Anna Luisa Costa[1] (1) – CNR-ISTEC, Institute of Science and Technology for Ceramics, National Research Council, Via Granarolo 64 Faenza, IT; (2) – Unit of Occupational Medicine, Department of Clinical and Experimental Medicine, University of Parma, 43026 Parma, Italy; (3) – Unit of General Pathology, Department of Biomedical, Biotechnological and Translational Sciences, University of Parma, 43025 Parma, Italy

The health impact assessment and control of nanostructures has been recognized to be part of the key areas of nanomedicine research [1,2]. The experience gained in the field of colloidal science has been fruitfully transferred to the control of nano particles (NPs) confinement and surface chemistry, opening new challenges towards the control of potentially adverse health impact of nanomaterials. This study is focused on the safety by design approach applied to AgNPs, usable as antibacterial surface agent, in order to mitigate their toxicity. At this purpose three synthesis procedures, implying three different surface modifying agents are presented. The treated AgNPs are in form of stable nanosols and the capped NPs have been synthesized by means of eco-friendly chemical methods easily transferable on large scale production. The used surface agents are the following: polyvinylpyrrolidone (PVP), sodium citrate and softcat SL30 (quaternary ammonium salt of hydroxyethylcellulose). The different electrostatic charge and steric hindrance of the three surface modifiers (Figure 1) produces consistent variations in the particles colloidal stability and chemical reactivity, affecting both the chemico-physical features and the biological behavior.

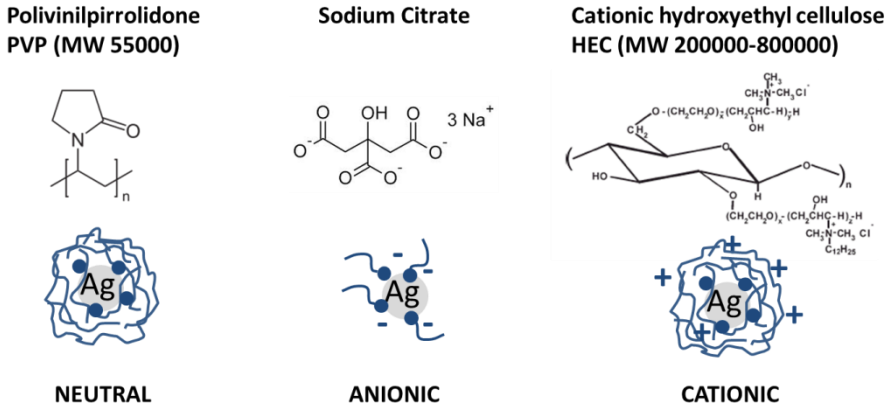

Figure 1_ Schematic representation of surface modifying agents steric and electrostatic contributes

The so-prepared capped NPs have been fully characterized by means of: DLS, TEM, SEM, XRD, Z-potential, UV-VIS and ICP. The results showed that the nanoparticles have spherical shape with diameters ranging from 10 to 40 nm according to the plasmonic resonance bands and different surface charge.

The cytotoxicity and the antimicrobial activity have been evaluated for all the modified samples showing relevant differences among the preparations.

Furthermore some risk determinant factors, such as the ionic fraction and the electrophoretic mobility, have been correlated to the biological reactivity, representing an important first attempt to support the safety by design approach.

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N° O7a-4

NANO CuO CASE STUDY: INTEGRATION OF SAFETY BY MOLECULAR DESIGN APPROACH

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The establishment of relationships between engineered nano particles (ENPs) physicochemical properties and principal factors determining an (eco) tox positive response passes through the colloidal characterization of nanomaterials in water and in (eco) tox relevant media (Figure 1), in order to better define their biological identity. The discovery of colloidal properties that drive directly the biological activity, improve the knowledge of nano-bio interaction mechanism with the concrete possibility to act on molecular design and drive any adverse biological effect (safer by design approach).
CuO commercial nanopowder (PlasmaChem GmbH) used as active component in the formulation of antimicrobial (preserving) wood coating, has been selected as case study. Colloidal properties as a function of double layer surface chemistry were assessed in order to provide necessary information for the control of biological reactivity in a Safety by molecular Design approach (SbyD). The following trends were observed:

- Trend vs pH; samples were dispersed at different pH (1);
- Trend vs surface adsorbed moieties. Three surface capping agents, differing for their charge (negative, neutral, positive) were added and let mixing with nanoparticles water suspension in order to promote the creation of self-assembled layers of additives on particle surface (2). neutral as well as positively or negatively charged, at increasing concentration.

Every experiment has been followed by characterization of the gained colloidal stability, expressed by Zeta potential determination, mean particle size and fraction of copper dissolved.
Results showed that copper oxides nanopowder without any stabilizing agent produce suspensions that are only slightly stable in the pH region close to neutrality, at basic pH these exhibit strong aggregation and subsequent precipitation, leading to poor transport fate mechanism and giving pollution of soils and sludge instead of superficial waters. On the other hand, acid solutions induce copper dissolution and therefore it is expected that the prevailing toxicity come from the dissolved fraction of Cu^{2+}. The experiments with surface coatings revealed that citrate ions (negative agent) adsorb on the surface, reversing starting positive zeta potential towards a plateau around -10mV. From corresponding size distribution data, the electrosteric stabilization of citrate does not seem sufficient to improve dispersability of the powder. The other two polymeric agents (PVP as neutral, B-PEI as positive) are capable of improving the colloidal stability by breaking the large aggregates. As part of SUN project, such key surface chemistry properties (acid base reactivity, surface coating), driving colloidal behavior in biological relevant media, will be related to (eco) tox factors In order to provide guidance for the development of a safety by molecular design approach.
The research leading to this commentary has received funding through the project "SUN" (NMP4-LA-2013-604305)

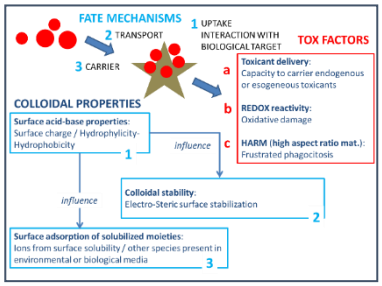

Figure 3 Colloidal properties affecting fate mechanisms and key (eco) tox determinant factors

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N° O7b-1

EFFICIENCY OF CURRENT ALTERNATIVES FOR PERSONAL DERMAL PROTECTION TOWARDS NANOHYDROSOLS

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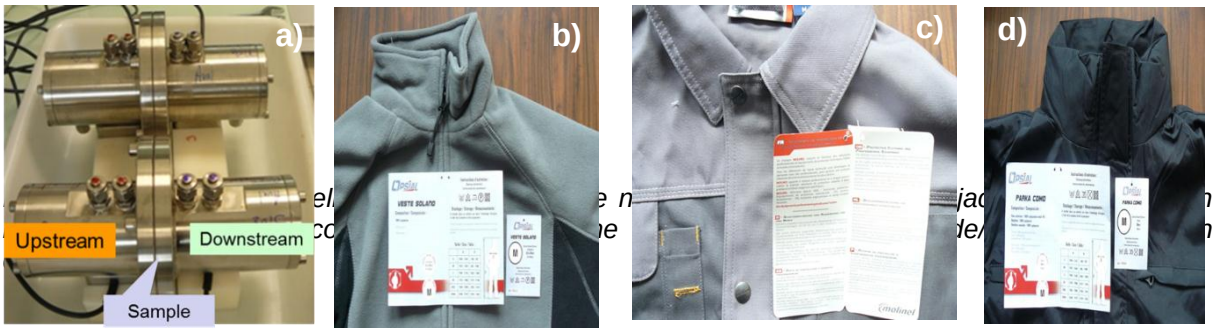
Manufactured NanoMaterials (MNMs) and nanocomposites are being considered for various uses in the construction industry. The use of NanoParticles (NPs) in construction materials is likely to increase because of highly valuable properties at low additive amounts. Consequently, there is an uncertainty with respect to health and safety risks and how to properly manage them to protect workers. The presented study was initiated within the frame of the FP7 Scaffold European project that aims to develop, test, validate and disseminate a new Risk Management Model. WP4 focuses on experiments testing effectiveness of PPEs and worker clothes in order to give conclusions on the composition of the different clothes to guide industrials in their choice. In this study, we tested the efficiency of different clothes used in the construction sector towards diffusion of SiO_2 NPs dispersed in water (hydrosol). The diffusion cell was composed of two cavities (upstream and downstream) separated by the cloth to be tested (see Figure 1 a)). Three types of clothes, i.e. non-woven (fleece jacket), woven (polyester 65%/cotton 35% material) and coated materials (Polyamide/Polyurethane) (see Figure 1 b), c) and d)), have been chosen due to their ability to resist to strong efforts and to evacuate the transpiration. We observed that the non-woven material was the least efficient toward liquid diffusion of SiO_2 NPs compared to the woven material. Indeed, the fleece jacket could swell with liquid and enhanced the diffusion of particles. For non-woven and coated materials, diffusion experiments took more time and kinetics were slower. We saw that the coated materials (PA/PU, rain coated material) was the most efficient because no NPs were observed in the downstream part of the diffusion cell. In conclusion, we observed that the thickness of clothes (the thicker the cloth is, the less NPs diffuse) and the weaving of clothes (woven materials cannot swell in liquid) reduced the NPs diffusion.



N° O7b-2

SECURED NANOMATERIAL WORKPLACES AT THE LITEN-PNS (CEA Grenoble - Platform Nano Safety) OPEN TO INDUSTRIALS, AS PRACTICAL CASE

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For several years, the research development involving nanomaterials led to preventive measures to move towards more specific and appropriate means, with the aim to reduce employee exposure to the lowest possible level. In the Serenade French LABEX program, safety work on laboratory equipment and research platforms at CEA Grenoble was finalized and opened to industrials as practical examples.

Corrective to preventive

At the beginning, the corrective

Many existing equipment used for the implementation of nanomaterials had to be modified and adapted to secure them to the nanomaterials risk.

The main issue was the risk containment. We need to adapt the collective protection equipment in our existing devices. These protection systems have been designed and made by integrating different issues (including operators, the type of NM and implemented phases, the process type, maintenance, quantities, the propensity of spread...). The fact that users were involved in the design allowed to validate the adequacy of protections in place.

To overall consideration, the prevention

Based on these early experiences, further pilot line platforms planned for implementation received improved prevention adaptation to take into account the Nano risk.

In assessing the risks functionally across different phases of implementations of nanomaterials, we were able to integrate “Prevention by Design”. The main ways of approach have conducted to the optimization of process phases (flow of materials, packaging, process, maintenance, etc.) to systematically reduce the risk of the different operations. In the same way, the design of pilot line platforms has integrated directly the safety on the different operating modes (taking into account nominal and degraded modes).

Part of the work performed at PNS (CEA Grenoble - Platform Nano Safety) is described in the

” Serenade” tour show (Figure 1), available for industrials.

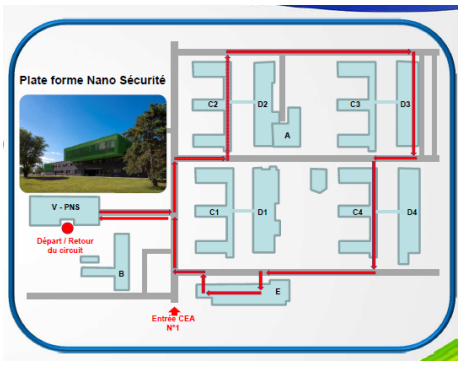

Figure 1: “Serenade” tour show exhibiting examples of nanosecured equipment at LITEN-PNS

N° O7b-3

NANOSECURED PLATEFORM TO ASSESS RISKS ALONG THE INDUSTRIAL LIFECYCLE OF NANOMATERIALS

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Nanotechnologies, as drivers of technological innovation, bring about new nanomaterials (NM) that may be associated with 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 they faculty 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) should also 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 its research and expertise. Located on the site of INERIS Verneuil-en-Halatte (France), the infrastructure of an area of 400 m², consists of four laboratories and associated facilities that are starting operating in August 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 the toxicity of NMs, it is a real challenge to setup experiments in which NMs are voluntary put into high energetic processes, e.g. to generate dispersed aerosols and dust explosion. Proper safety barriers and containment devices are being implemented so as to enable the safe use of safety test apparatuses initially designed for non toxic micrometric powders, such as the ones used to assess the consequences of accidental scenarios (fire, exposure) in the laboratory itself, without generating an accident.
In this paper, the main laboratories and pertaining safety tests of this platform will be described, together with the proper associate safety barriers and measures. From experimental observations, we will practically illustrate how this platform can help improving the safety of some typical industrial processes. The link with the work under progress within CEN TC 352 WG3 on the definition of "Protocols for determining the explosivity and flammability of nanopowders (for transport, handling and storage)" will be presented.

Acknowledgments: This platform as being designed as a research and innovation tool in Picardy is financially supported by the Regional Council of Picardy who participates up to one million Euros in the project.

N° O7c-1

TOWARD UNDERSTANDING THE MECHANISMS AND THE KINETIC OF NANOPARTICLE PENETRATION THROUGH PROTECTIVE GLOVES

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Parallel to the increased use of engineered nanoparticles (ENP) in the formulation of commercial products or in medicine, many Health & Safety agencies have recommended the application of the precautionary principle to handle ENP namely the recommendation of the use of protective gloves against chemicals. However, recent articles underline the penetration of titanium dioxide nanoparticles through nitrile rubber protective gloves in conditions simulating occupational use.
This project is designed to understand the mechanisms and the kinetic of ENP penetration through protective gloves in work conditions. The first step of this study is to find correlations between the penetration of ENP through protective gloves and the mechanical and physical behavior of the elastomer material subjected conditions simulating occupational use.
For this work, two types of nitrile rubber gloves (NBR-100 and NBR-200, 100 µm and 200 µm thick respectively) were brought into contact with 5 nm and 50 nm gold nanoparticles in water (labeled as nAu-5 and nAu-50 respectively). 30mm-biaxial dynamic deformations (BD), simulating the flexing of the hand, were applied to the samples during their exposure to ENP.
The efficiency of the protective gloves in contact with gold nanoparticles and subjected to BD is evaluated with the gold concentration measuring in a saline solution used as a sampling solution. The gold concentration is obtained by ICP-MS (Inductively Coupled Plasma – Mass Spectrometry) and the size distribution by NTA (Nanoparticle Tracking Analysis). The first results show that the gold concentration increases after 3 hours of BD (1 BD each 30 seconds) for NBR-100 samples (Figure 1). At the same time, significant changes have observed on the outer surface of the glove sample. Indeed the number and the surface of the micropores on the surface increase. Moreover, BD affect mechanical properties (strain energy) of protective materials. On the other side, nitrile rubber protective gloves are also shown to be sensitive to the action of ENP solutions and to the action of the saline solution used to simulate sweat.

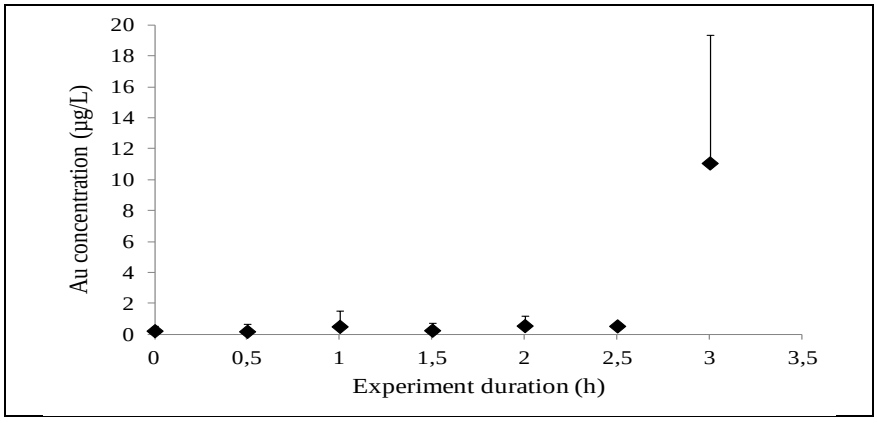

Figure 1. Gold concentration penetrating nitrile rubber glove samples (100 µm thick) exposed to colloidal solution of nAu-5 and 30mm-BD

N° PL8

THE LIFE CYCLE PERSPECTIVE AS BASIS FOR ASSESSING ENVIRONMENTAL RISKS OF ENGINEERED NANOMATERIALS

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The current and future usage of engineered nanomaterials (ENM) in industrial applications and consumer products will cause emissions of ENM to the environment and thus result in environmental exposure. As a starting point for an exposure assessment, exploring sources and pathways of release helps to identify relevant applications and release scenarios. By tracking the life cycle of products, it is possible to quantitatively predict the flows of ENM to the environment. 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 limited information on the characterization of the released ENM is available. This presentation gives an overview on the most recent progress in modeling the flows of ENM to the environment, extending the models to new materials (e.g. nano-gold and nano-SiO_2) and incorporating dynamic aspects of production and use. The newest predicted environmental concentrations are then used to derive an updated environmental risks based on probabilistic effect assessments, incorporating an up-to-date evaluation of environmental effects. A necessary step in any nano-risk assessment is the comparison of the nano-flows with those of the conventional materials. Results will be shown for both the exposure as well as the hazard side.

N° O8a-1

NANOMATERIALS IN CONSTRUCTION AND DEMOLITION – HOW CAN WE ASSESS THE RISK IF WE DON'T KNOW WHERE THEY ARE?

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Construction is an important field for the use of nanomaterials. The high volume of concrete, steel and glass used worldwide; the drive to reduce the environmental impact of building processes; and the opportunities to improve energy efficiency in homes and workplaces create a huge potential market for novel products. However, construction is historically conservative, driven by cost constraints and the need for predictability in materials. Concerns about health risks from new products are a further possible inhibitor to development; but there is also a risk that products are developed without potential health implications being considered. Any potential for harm must be managed throughout the life cycle of the built environment, much of which takes place outside of easily controlled work environments.

Establishing exactly where nanomaterials are used in construction is difficult. Several overview papers have been written e.g. [1, 2] but these rely largely on published academic literature and the limited data available from manufacturers, resulting in uncertainty. For example, the reported potential to enhance concrete by adding 1% carbon nano tubes (CNT) has led to anxiety in some quarters; unsurprisingly given the apparent potential for CNT to adversely affect the body in similar ways to asbestos [3]. Yet there is little evidence that such products have found their way from research laboratories to the market, largely due to current high costs and low production volumes of the raw materials. A further complication is that some manufacturers use a nano 'badge' as a marketing tool, or to label products developed through the use of nanotechnology rather than those using nanoparticles.

Research funded by the Institution of Occupational Safety and Health (IOSH) has started to assemble a catalogue of construction products found in the European marketplace which contain or might contain nanomaterials and gathering samples of each. Tests using Thermal analysis, Raman and Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy, and X-ray diffraction will identify which are genuinely nano-enabled. Tests will also characterise any nanomaterials found including chemical composition, size and dispersion. Detail of this nature is important, given the wide variation in potential health effects between different forms of chemically identical nanomaterials [4].

The research will then consider demolition and recycling routes for construction materials, assess the potential for release of nanomaterials through these processes and determine any potential health risk. The outcome will be pragmatic guidance for construction indicating where particular protection measures may be appropriate and providing reassurance where no action is required.

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N° O8a-2

NANOMATERIALS IN CONSTRUCTION AND DEMOLITION WASTE IN SWITZERLAND

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Nanomaterials (NMs) used in the construction industry will enter recycling and construction waste through renovation and demolition of buildings. Information about NM flows in these processes is insufficient, thus the potential release of NMs into the environment and the implications of this release are unknown. To obtain information about NM applications and amounts used in construction industry, we surveyed representatives from the Swiss construction industry. Although the survey revealed a lack of knowledge about the application of NMs in construction, it showed that NMs are mainly used in paints and cement. The most frequently used NMs in construction are nano-titanium dioxide (nano-TiO_2), nano-silicon dioxide (nano-SiO_2), nano-zinc oxide (nano-ZnO) and nano-silver (nano-Ag). We complemented this information with literature data and market reports to estimate the mass content of NM-containing construction products and to calculate the amount of paint and cement sold in Switzerland for the year 2012. With a top down semi-quantitative approach, we estimated the flows of NM contained in paints to recycling and landfills. The results show that waste from paint is contained in materials like concrete, bricks, gypsum or wood and the flows of the NM are determined by the flows of these building materials. The main amounts of NMs contained in paints will enter the recycling system, followed by incineration and landfill. The amount of NM in construction waste coming from paint would be less than 1 ton in a year in Switzerland, with nano-TiO_2 and nano-SiO_2 being the most relevant NMs. The potential for release of NMs is high during recycling, so we recommend to place special emphasis in the future on this process due its importance for occupational health. Although the amount of NMs going into landfills seems relatively small, further analysis is required to determine the release potential to environmental compartments from these facilities.

N° O8a-3

FLOWS OF ENGINEERED NANOMATERIALS THROUGH THE RECYCLING PROCESS IN SWITZERLAND

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The use of engineered nanomaterials (ENMs) in diverse applications has increased during the last years and this will likely continue in the near future. As the number of applications increase, more and more waste with nanomaterials will be generated. A portion of this waste will enter the recycling system, for example, in electronic products, textiles and construction materials. The fate of these materials during and after the waste management and recycling operations is poorly understood. The aim of this work is to model the flows of nano-TiO_2, nano-ZnO, nano-Ag and CNT in the recycling system in Switzerland. The basis for this study is published information on the ENMs flows on the Swiss system. We developed a method to assess their flow after recycling. To incorporate the uncertainties inherent to the limited information available, we applied a probabilistic material flow analysis approach. The results show that the recycling processes does not result in significant further propagation of nanomaterials into new products (*Figure 1*). Instead, the largest proportion will flow as waste that can subsequently be properly handled in incineration plants or landfills. Smaller fractions of ENMs will be eliminated or end up in materials that are sent abroad to undergo further recovery processes. Only a reduced amount of ENMs will flow back to the productive process of the economy in a limited number of sectors. Overall, the results suggest that risk assessment during recycling should focus on occupational exposure, release of ENMs in landfills and incineration plants, and toxicity assessment in a small number of recycled inputs.

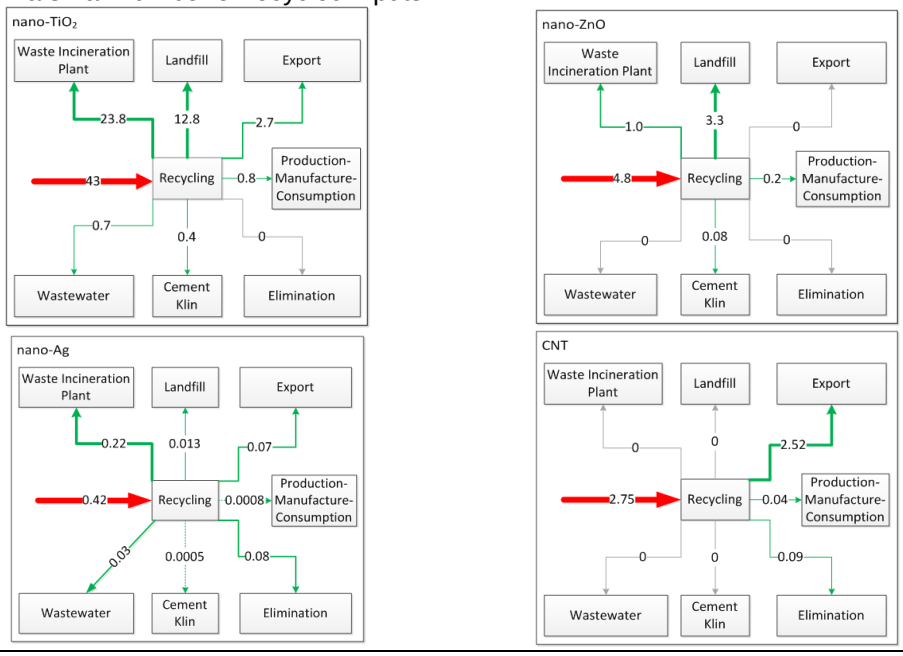

Figure 1. *Mass flow diagrams for each ENM type from the recycling system to other compartments. The red arrows represent the inflows to the recycling system while the green ones represent the outflows. The numbers are the modes (tons/y) of the flow distributions in 2012.*

N° O8a-4

ENVIRONMENTAL IMPACTS OF MULTIWALLED CARBON NANOTUBES (MWCNT) AND PLATINUM IN FUEL CELL TECHNOLOGY

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Producing energy with water as only direct emissions is an attractive concept behind fuel cells. The expensive platinum catalyst has plagued the design of fuel cells because the environmental and economic performance is diminished by the high platinum demand. Replacing carbon black with multiwalled carbon nanotubes (MWCNT) allows reducing the amount of platinum by enlarging the catalytic active surface. The aim of this work was to use life cycle assessment to compare the environmental performance of two Fuel Cell (FC) systems, a conventional electrocatalyst using carbon black as supporter for the platinum and an electrocatalyst using MWCNT as supporter for the platinum. Industrial data was collected for the MWCNT production (Nanothinx, Greece) and the production of the electrocatalyst, while all other components of the FC rather refer to a technically sensible option than to a specific product. Environmental impacts were evaluated with ReCiPe. First results demonstrate that platinum causes most environmental impacts in a FC unit (90% all of environmental impacts), even though its mass (0.2%) is irrelevant. The production of the MWCNT as supporter for the platinum is connected with higher environmental burden than the production of carbon black. However, this difference is overcompensated by far with the platinum savings which leads to an overall benefit for the FC unit with MWCNT of 21% compared to the FC with CB as supporter.

***Figure 1**. Production, disposal including benefits due to recycling and overall environmental performance for both types of fuel cells. The damage is depicted relative to the damage of the components containing MWCNT.*

N° O8a-5

LIFE CYCLE BASED SOCIO-ECONOMIC ASSESSMENT COMBINING ENVIRONMENTAL IMPACT, OCCUPATIONAL HEALTH RISKS AND HEALTH BENEFITS FOR NANOSILVER COATED DOOR HANDLES

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Because of hospital infections patients may need additional medical treatment or even decease. One of the causes are microbes. These microbes live on surfaces in the hospital, and can be transferred by hand contact. Anti-microbial surface coatings may reduce the number of these hospital acquired infections (HAI).
This study examines an anti-microbial nanosilver-polysiloxane coating applied by hand on steel door handles in hospitals. The examined system contains all precursors requested to produce the nanosilver-polysiloxane coating (i.e. starting from the extraction of the resources), as well as all subsequent life stages after the application up to the final disposal of the coating on the door handle. The coating is re-applied every two years to overcome the wear of the coating. The production and disposal of the door handles themselves are excluded in this study. Although health benefits are expected the handling of nanosilver may also cause an occupational health risk.
To assess the environmental impact, the ReCiPe method was combined with impact category specific shadow prices. In this way the results can be displayed in a common unit: the euro. The impact of nanosilver emissions on occupational health was assessed with an exposure-hazard model. The specific health effects were expressed as DALYs and these were translated into euros. This allows the occupational health results to be combined with the environmental impact from the LCA.
The expected benefit of the nanosilver coating is a reduced number of HAI. The reduction of HAI and the economic benefits of this coating were estimated based on literature Three scenarios (Optimistic, Realistic and Conservative) were established, to take uncertainty into account, in e.g. number of door handles per hospital or number of infections prevented.
This study shows that silver emissions to soil and air cause over 30% of the total environmental impact. The largest part by emissions to agricultural soil. This affects both Human Toxicity as Terrestrial Eco-Toxicity.
However, the contribution of the environmental impact and also that of the occupational health risk are almost insignificant compared to the costs of the coating application. The benefits of reducing the number of HAI are shown to outweigh the total of the socio-economic costs for every scenario.

N° O8a-6

LCA-INTEGRATED HUMAN HEALTH RISK ASSESSMENT: APPLICATION IN FOUR CASE STUDIES ON ENM

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Nanomaterials have great market potential for SMEs, but the introduction of nanomaterials is hampered due to the unknown human and ecological risks. For this reason, guidance is needed to assess the risks and the benefits of the nanomaterial in their products in comparison with the conventional (non-nano) products. The major characteristics of regulatory risk assessment (RA) are its substance- and population specificity, that it is focusing on only two stages of the product life cycle (LC), i.e. workers and consumers, and it usually neglects exposure of man via the environment. The human health RA within Life Cycle Assessment (LCA), aggregates all substances emitted to an environmental compartment, however, it neglects exposure during production and use of products. The most important difference, however, is the metrics used to indicate the risk. Regulatory RA results in risk characterization ratios to demonstrate (un) safety, whereas LCA expresses risk rates, e.g. health end-points per unit of product or functionality. The latter enables comparison of the impact of different products. The LICARA projects aimed to develop methods to aggregate risks across the entire LC of the product in combination with 'conventional' LCA assessment and its impact parameters. Therefore, workers and consumers exposure have been integrated in the LCA approach. The concepts of such an LCA-'integrated' RA, which consists of a basic level RA for all LC-stages, have been developed. It aggregates RA over life cycle stages and include indoor exposures. The major challenge was to test the feasibility of these concepts and more specifically the metric of the RA outcome, e.g. DALY/ kg or unit of functionality, which should be aligned with LCA to enable comparison. The testing of the proof of principle has taken place in four case studies to balance the human health RA within LCA of nano-enabled products. The concepts of this LCA-integrated RA approach and results of the case studies will be presented.

N° O8a-7

LICARA NANOSCAN: EVALUATING BENEFITS AND RISKS OVER THE LIFE CYCLE OF NANOPRODUCTS

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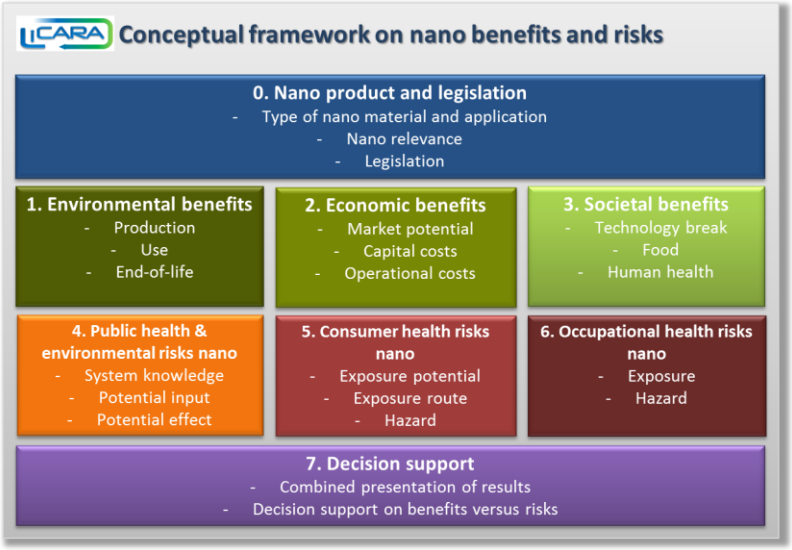
The EU FP7 project LICARA (Life Cycle Approach and human Risk Assessment) aims to develop a framework to assess the sustainability of nanoproducts. Nanoproducts may pose risks for human (consumers, workers and/or the public) and the environment but also can offer great economic, environmental and social opportunities. In order to avoid a shift of undesired effects to elsewhere or later (a definition of being “sustainable”), it is important to evaluate the risks and benefits of products over their complete life cycle, i.e. from production till end of life.
Currently there are various tools and methods to determine the risks of nanomaterials taking into account a variety of aspects such as toxicity, emissions, fate and exposure (Stoffenmanager Nano, Precautionary Matrix, NanoRiskCat, NanoTool, ANSES, NanoGuidance). However, none of the systems gives information about the benefits of the nano-enabled product. Within LICARA, a conceptual framework is developed to scan the risks and benefits of nanoproducts in a structured way. It combines the main principles of Multi-Criteria Decision Analysis, Risk Assessment and Life Cycle Assessment and presently available scientific insights on the different aspects of nanomaterials and -products. In order not to reinvent the wheel, a selection of existing tools is used to determine the risks of nanoproducts over all stages of the life cycle. Within the framework, these are extended with assessment of benefits.
At present, information on the risks and benefits of nanomaterials is scarce. To repair data gaps on the short term is costly if not impossible. In order to facilitate SMEs with limited means and time in performing an evaluation of pro’s and con’s of a nanoproduct, a prototype tool has been developed. It is named LICARA nanoscan and supports the user in executing all different evaluation steps, translating current state of the art know-how in understandable information and evaluating the results.



N° O8a-8

HUMAN TOXICITY AND FRESHWATER ECOTOXICITY CHARACTERISATION FACTORS FOR ENGINEERED NANOPARTICLES: TOWARD A SPATIAL DIFFERENTATION

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Engineered nanoparticles (ENPs) are increasingly used in several applications, from medicine to environmental remediation. In the last decade several concerns on their potential human and environmental impacts have been raised. ENPs release into the environment may occur throughout their entire life cycle: from production to the fabrication of engineered nanomaterials (ENMs) containing ENPs, to the use and end of life phase of those products. Hence, Life Cycle Assessment (LCA) has been addressed as a systematic method to determine the human and environmental impacts of nanotechnology products at several stages of their life cycle. In spite of this only a few LCA studies on nanotechnology and on ENPs have been published to date and, even fewer assessed the human and ecotoxicological impacts. Currently, the knowledge gaps in the field of risk assessment of nanotechnology are reflected in LCA, where characterisation factors (CFs) for toxic impact categories are missing. The determination of characterisation factors requires the knowledge of the environmental fate of a substance, human and environmental exposure and its toxicity. Within the Life Cycle Impact Assessment (LCIA) for the no-toxic impact categories (e.g. Climate change), no special difficulties in the assessment of nanoproducts can be foreseen. On the other hand, for toxic impact categories, the current knowledge on the toxicity of ENPs and on their environmental fate, exposure and toxicity may not be sufficient for a representative characterization of nanoproducts. The present research is focused on LCIA with the aim of presenting the possibilities of CF calculation for metal oxide nanoparticles (as n-TiO_2), for the impact categories of human toxicity and freshwater ecotoxicity and considering spatial differentiation. The framework proposed is based on the USEtox model. Morover, it applies the recent multimedia environmental model (SimpleBox4Nano) developed by Meester et al. 2014 to calculate the fate factor for n-TiO_2 considering both air and freshwater comparents. Thus, specific-fate process for ENPs (attachment, aggregation, dry deposition ect.) have been accounted and described by first order kinetic rate. Whereas, the human and environmental exposure have been calculated following the method proposed by USEtox framework. The fate and exposure factors to ENPs are evaluated accounting several geographical scale (continental, urbans) and spatial archetypes (Sala et al., 2012). In fact within the fate calculation the systemic dimensions (e.g *area, height, volume of atmosphere, soil and water)*) and (*ENPs radius, ENPs mass density, aggregation efficienty and attachement efficienty*) have been assessed to different spatial scale. Hence, the present study allows to calculate the CFs for human toxicity and freshwater ecotoxicity for n-TIO_2 accounting for different spatial scale.

N° O8a-9

FRAMEWORK FOR HUMAN HEALTH CHARACTERIZATION FACTOR CALCULATION OF TIO_2 NANOPARTICLES

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The widespread use of engineered nanoparticles (ENPs) in various industrial applications is leading inevitably to releases of these materials into the environment, increasing like this human and environmental exposure to these substances, and is getting consequently more and more a concern regarding their potential adverse effects on both, the environment and the human health. In this context, Life Cycle Assessment (LCA) is recognized as one of the key methods for the assessment of the environmental performance of products containing such ENPs. However, so far, factors to assess releases of ENPs into the environment have been still completely missing, making all LCA studies of such materials incomplete. For this, a clear toxicological characterization of the effects is a prerequisite in order to establish trustworthy characterization factor (CFs) for release of nanoparticles into the environment. Humans could potentially be exposed to ENPs releases along the whole life cycle (i.e. during manufacture, handling, use and disposal treatment of ENPs). Therefore, this work aims to provide a methodological framework for establishing human health CFs for releases of ENPs, using titanium dioxide nanoparticles (TiO_2-NPs) as an exemplary example. Starting point for this is USEtox, the internationally recognized consensus model for the assessment of toxicity within LCA studies. USEtox calculates the impact on human toxicity as product of emission, intake fractions (iF) and effect factors (EF). The intake fraction is originally defined as ratio of the mass intake by an individual over the mass released to the environment. The effect factor on the other hand contemplates the change in life time disease probability due to change in life time intake of a pollutant. Both effects, i.e. carcinogens and non-carcinogens, are taken into account in the calculation of the actual EF:

- the EF for carcinogens effects is determined based on a benchmark dose used by the National Institute for Occupational Safety and Health (NIOSH) to determinate the recommended occupational exposure limit (REL) for TiO_2-NPs;
- the EF for non-carcinogens effects is calculated based on NOAEL (no-observed adverse effect level) and LOAEL (lowest observed adverse effect level) values.

Limiting the examinations here on releases to air as only investigated compartment, a one-box model using steady-state conditions and direct human exposure can be applied for the calculation of the intake fractions. Here, intake fractions for indoor and outdoor conditions have been calculated for TiO_2-NPs. While indoor iF, a complete mixing for the volume and the indoor volume per workers for the Chemical industry in Switzerland have been evaluated. For the outdoor iF, the fate factor matrix has been calculated by applying the SimpleBox4Nano (SB4N) multimedia modelling developed by Meesters and co-author. Thanks to this model it is possible to obtain transport and removal rates constants for ENPs in and across air, rain, surface water, soil and sediment compartments, taking into account various input parameters (i.e. radius, mass density, aggregation and attachement efficiency of TiO_2-NPs) and systemic dimensions (area, height and volume for each compartments). Again, based on the study by Mueller and Nowack, the scenario is focusing on Switzerland as geographical area.

The resulting CFs for indoor and outdoor environment and for carcinogens and non-carcinogens effects of TiO_2-NPs are the following:

Human Health effect	CF_{indoor} [cases/$kg_{emitted}$]	$CF_{outodoor}$ [cases/$kg_{emitted}$]
Carcinogenic	5.93E-04	1.55E-05
Non-carcinogenic (NOAEL)	7.04E-06	1.84E-07
Non-carcinogenic (LOAEL)	3.52E-04	9.18E-06

N° O8a-10

TOXICITY CHARACTERIZATION FACTORS FOR NANOMATERIALS: CURRENT DEVELOPMENTS AND LIMITATIONS

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The potential application of engineered nanomaterials (ENM) in areas such as biomedicine, electronics or energy storage has increased significantly their production and use. At the same time, it has also increased public concern regarding ENMs' potential effects for both humans and the environment. In order to evaluate these possible impacts, we first need to calculate the expected emissions of ENM through their life cycle. Later, once the ENM reaches a compartment (air, soil, water), we have to assess its behavior in the environment. Finally, if ingested by a human or another animal, we must quantify its effects.

The Comparative Assessment of Toxic Emissions (CATE) is a branch of Life Cycle Assessment (LCA, Fig.1). It allows us to rate the potential impact of a chemical linking its behavior in the environment (its fate), and its harmful effects. These impacts are expressed as characterization factors (CF). USEtox is a consensus methodology for CATE currently including more than 3000 substances in its database. ENM are still not part of this database but several researchers have already applied USEtox to calculate CF for ENM such as carbon nanotubes (CNTs), nanoAg, and nano TIO_2. We reviewed existing CF for ENM and calculated our own CF for CNTs. We selected a single-walled and a multi-walled CNT (SWCNT and MWCNT respectively) both of 13 nm in diameter and 1µm in length. Current values suggest that CNTs are not a homogeneous group in term of toxicity and that MWCNTs could be more toxic than SWCNTs for both humans and aquatic life (Fig 2.).

The reliability of LCA, and thus of CATE, depends strongly on data quality. Using CNTs as example, we will assess the parameter uncertainties affecting the calculation of CF for ENM. Model uncertainty also affects CATE of ENM. General methodologies such as USEtox do not cover all the particularities of ENM behavior in the environment such as aggregation or dissolution. Contrarily, nano-specific methodologies such as SimpleBox4nano currently assess only the fate of these substances and not its potential toxic effects. We aim at combining both methodologies so future CF of ENM can more accurately describe the potential effects of ENM.

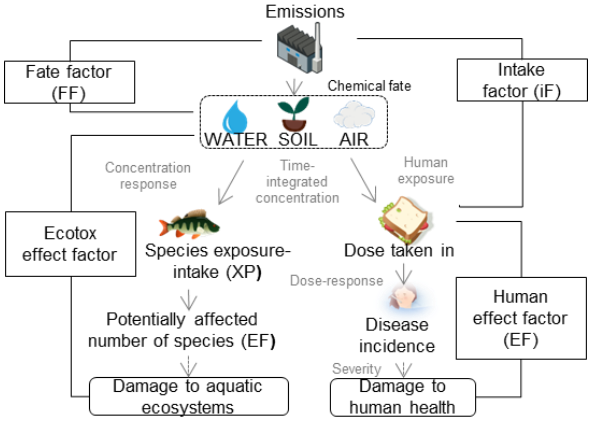

***Figure 1**. Framework for Comparative Assessment of Toxic Emissions, adapted from Hauschild et al. Env. Sci. Tech. 42 (2008) 7032*

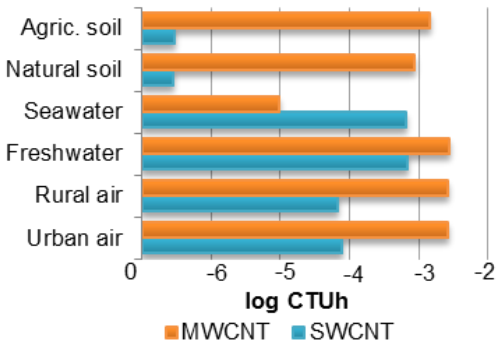

***Fig. 2.** Human Toxicity of two kinds of CNT*

N° PL9

REGULATION PERSPECTIVES

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Nanotechnologies are a rapidly developing field of science, technology and innovation. It involves the development and manufacture of materials structured at the nanometer size range, which includes the production and use of nano-objects, "so called nanomaterials for some regulators". It should be stressed that nanomaterials is a material categorization by the size of its internal or external structure which will present new specific properties.

Major implications of nanotechnologies are expected in areas such as health care, information and communication technologies, energy production and storage, materials science/chemical engineering, manufacturing, environmental protection, consumer products, etc.
The world market for nanotechnologies will increase fast and dramatically, and they are expected to provide a significant input to the creations of manufacturing jobs world-wide.
On the other hand, nanotechnologies and nano-objects 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. It does not imply a specific risk, nor does it necessarily mean that these materials have new hazard properties compared to their constituents, but they may be the source of concerns with regard to risks caused by their specific properties.
Given the interests at stake, the European Commission has set out a European Strategy for Nanotechnologies, based on a «safe, integrated and responsible» approach.One of the building blocks of the "safe, integrated and responsible" approach is Standardization (soft regulation). 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 (hard) regulation.
At different levels, policies to this end are being undertaken. More and more specific "nanomaterials" regulations take place at national (Belgium, France, Denmark,..) or European (Reach, Food, Cosmetic,…) level to protect customers and citizen environment from the uncertainties regarding the risk assessment and safety evaluation of nanomaterials.

N° O9a-1

NANOMATERIALS IN FOOD
CURRENT AND FUTURE APPLICATIONS AND REGULATORY ASPECTS

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Nanotechnology can contribute to the development of innovative applications in the food and feed sector with new and enhanced properties: increased delivery of nutrients and efficacy of pesticides by nano-encapsulation, altered colour, flavour, texture of food components or nano-sensors for traceability and monitoring of food conditions during transport and storage. It is expected that these applications will increase in the future and thereby represent a relevant source of direct exposure of humans to nanomaterials (NM). To address this potential concern and to gain more up-to date information, RIKILT and JRC have prepared for the European Food Safety Authority (EFSA) an inventory of currently used and reasonably foreseen applications of NM in agriculture and food/feed production.
The inventory contains information received through an extensive search of literature and webpages, complemented by questionnaires sent to different stakeholders. An analysis of the information records in the database shows that nano-encapsulates, silver and titanium dioxide are the most frequent type of NMs listed and that food additives and food contact materials are the most frequent type of applications. Most of these applications (more than 80 %) are actually making use of only 20% of different types of NM recorded. A comparison between current and future applications indicates a trend from inorganic materials (e.g. silver) towards organic materials (nano-encapsulates, nanocomposites). Applications in novel food, feed additives, biocides and pesticides are currently mostly at a developmental stage.
The review of EU and non-EU legislation shows that certain EU legal acts incorporate a nanomaterial definition. Besides a definition specific for NM in food (Regulation 1169/2011 on food information to consumers) and plastic food contact materials (Regulation 10/2011) a Recommendation (2011/696/EU) for a broadly applicable definition across different regulatory sectors is available. In the EU nanotechnology applied to food is regulated by the Novel Food Regulation (No 258/97) which is currently under revision. A pre-market approval (safety assessment and authorization) is required for novel food and food additives. A separate risk assessment for the nanoforms is required under several EU frameworks. Food additives already on the market which may contain NM fractions are subject to re-evaluation in the EU, during which potential nanospecific data can be considered. As of December 2014 food will have to be labelled for its content of NM.
Countries outside the EU have limited NM specific legislation and no legally binding definition. They rather adopted a broader approach which builds mainly on guidance for industry.
The presentation will give an overview of current and future NM applications in the agri/feed/food sector and discusses possible implications for the safety of humans. It will also examine differences in EU and international regulation of nanotechnology in food.

N° O9a-2

CLASSIFICATION AND REPORTING OF NANOSTRUCTURED SILICA MATERIALS

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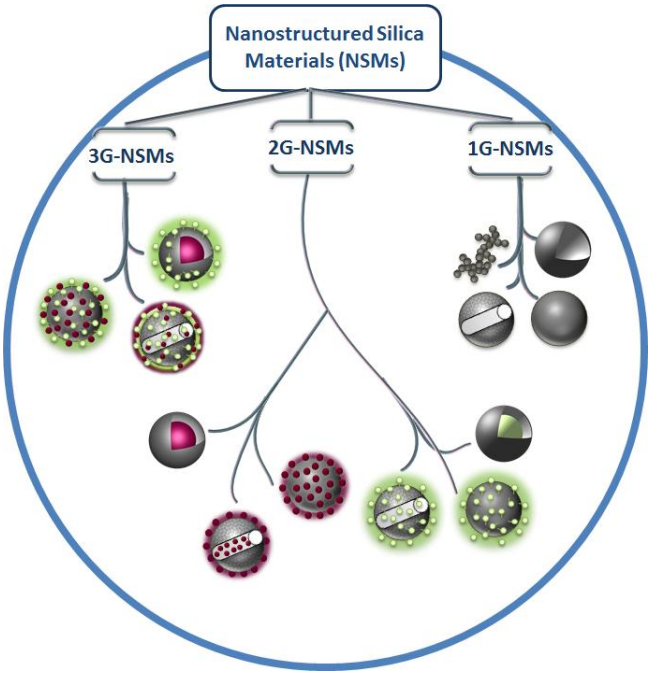
Concerns about identification and registration of nanomaterials have been raised due to the risk of possible health effects posed by them. EU legislation in general covers regulations of nanomaterials, but the extent of information required for substance identification and reporting needs is not clearly defined.
Nanostructured silica materials (NSMs) as an example, we highlight the importance of not only composition but also the structural complexity of NSMs for their identification and registration. We reviewed various forms of NSMs and for the first time, recommend a classification system by their composition, extent and location of surface treating agents. Bare silica nanoparticles are grouped to 1st generation NSMs (1G-NSMs), nanocomposites of silica with organic or inorganic as their secondary phase are in the 2nd Generation NSMs (2G-NSMs) and finally, nanocomposites of silica comprising both organic and inorganic as their counterparts as 3rd Generation NSMs (3G-NSMs). The proposed classification enables to identify various NSMs by their complexity and helps to define the requirements for risk and hazard assessment. In addition, we have identified volume specific surface area (VSSA) as an additional identifier, and proposed a scheme for identification and reporting of NSMs. We argue that VSSA is also important to define their origin and properties by association of organic and inorganic compounds.
Our methodology of NSMs will enable scientists, industrialists and regulators to identify the nanomaterials systematically and guide them to identify their unique properties towards risk and hazard assessment.



Classification of Nanostructured silica materials (NSMs) by their composition and structural complexity associated with organic (green)/ inorganic compounds (Purple).

N° O9a-3

INTERNATIONAL AND EUROPEAN STANDARDIZATION IN NANOTECHNOLOGY; HOW STANDARDIZATION CAN HELP INDUSTRY AND REGULATORS IN DEVELOPING SAFE PRODUCTS?

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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. ISO and CEN have respectively started in 2005 and 2006 to deal with selected topics related to this emerging and enabling technology.
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 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 Phys. Chem. characterization of nano-objects and Exposure to nanomaterials and any matters related to Health, Safety and Environment.

Answers will be given to: How it works? Where are we right now and how work is going from now onwards? How CEN’s work and targets deal with and interact with global matters in this field?

N° O9a-4

NATIONAL NANO REGISTERS: ADMISSIBILITY UNDER EU LAW AND REGULATORY UNCERTAINTIES

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Until now, the European Union institutions have favoured a sector-based approach to address alleged safety concerns about the inclusion of nanomaterials in consumer and non-consumer goods. These policy options have been materialized in the new cosmetics, biocides and food regulations which contain special provisions relating to nanomaterials.
For several years, some member States and stakeholders have called for further EU action in the field of health and environmental protection. In particular, they suggest either amending the REACH regulation or adopting a new regulation to control the market placement of nanomaterials. Other member States and stakeholders are reluctant to open a Pandora's Box that could lead to overregulation and subsequently impact upon the competiveness of the nano industries.
The absence of consensus at the EU level has led to an uncommon situation whereby three member States have created national nano registers independently of any EU initiative. France has been the first State in the world to adopt a nano register that came into force on 11th January 2013. Denmark has also had a national register in place since 18th June 2014 and Belgium will have one by 1st January 2016. Sweden and Italy are currently assessing the opportunity of having their own register and other member States may follow. In parallel, the European Commission has published a study and organized a public consultation on the opportunity of revising REACH or adopting an EU-wide nano register.
The adoption of national registers outside of the EU framework has created a precedent that could render the EU nanotechnology policy obsolete. It has also left stakeholders with many outstanding issues, notably the admissibility of such registers under EU law.
Indeed, these national nano registers restrict the free movement of goods within the European internal market. Such restrictions could constitute a violation of the Treaty on the Functioning of the European Union if they cannot be justified in the light of the treaty itself.
There are also regulatory uncertainties about these national nano registers. Firstly, their scope is determined by the definition given to nanomaterials and other chemicals subject to registration requirements. There are certain grey areas surrounding these definitions as they could either catch more chemicals than expected or, on the contrary, be misinterpreted by companies which would not make any filling with the national administrative authority and therefore be exposed to penalties for non-compliance with the registration requirements. Secondly, it is uncertain whether national authorities will effectively use the technical details disclosed in the fillings to conduct inspections or even forbid the use of certain nanomaterials which they consider to be hazardous. The multiplication of national registers exacerbates the risks of having different nanotechnology policies within the European internal market, thereby exposing companies to excessive administrative burdens.

N° O9a-5

PAVING THE WAY FROM RESEARCH TO STANDARDS IN THE FIELD OF NANOTECHNOLOGIES: THE NANOSTAIR SUPPORT AND PRE-NORMATIVE WORK.

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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. It contributes to sustainable competitiveness through "reference documents". Standardization is particularly important in the field of nanotechnologies where knowledge and products make rapid progress. In this field especially, the efficient transfer from research to standardization is essential.
Different barriers currently limit this transfer and thus a part of the proper exploitation of research outcomes. Among these barriers: the lack of resources for standardization after the R&D projects, and some difficulty to enter into the standardization process. On the other hand, researchers are eager to share their methods and have them recognized as standards.
As a response to these barriers, the FP7 cooperative action nanoSTAIR (www.nanostair.eu-vri.eu) has built a sustainable process and platform in the field of nanotechnologies to support the transfer of knowledge from research to standards. Running from September 2012 to February 2014, it was operated by a consortium gathering national and European research institutes and standardization bodies. The nanoSTAIR team continues further its activities.
The nanoSTAIR project has set-up a mechanism to identify the opportunities for standardization in the results of research projects, based on the semantic analysis of research papers and on expert review. For selected results, nanoSTAIR pools together resources and consortia sharing similar standardization opportunities and provides assistance for selecting the right standardization umbrella (CEN or ISO Technical Committee and Working Group) and for launching new standardization work items. 13 research documents have gone through the semantic analysis ("nanoSTAIR check"), and 2 research results have been selected and are being transferred into new standards by the research teams with nanoSTAIR support.
nanoSTAIR promotes the early implementation of this approach, from the very conception of research projects: this would allow an interlaboratory work on protocols within research projects (sharing, training, adjusting, comparisons, round robin,…) more consciously and optimally organized as a pre-normative work, thus dramatically facilitating the further last step to standardization - and this with minimum extra work.
The whole process will be illustrated with the case study of the new work items selected by nanoSTAIR and by the complementary case study of pre-normative work on protocols within the FP7 project QualityNano, together building a complete -and still ongoing- story from the start of a research project to the entrance to standardization.

Acknowledgment: The nanoSTAIR and QualityNano research has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreements n° 319092 and n° 262163.

N° O9a-6

THE nanoSTAIR STRATEGY: A NEW STRATEGIC PROPOSAL TO IMPULSE STANDARDIZATION IN NANOTECHNOLOGY RESEARCH

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Nanotechnology is considered one of the key technologies of the 21st century within Europe and a Key-Enabling Technology (KET) by H2020. The production of knowledge in nanotechnology is very intensive as a great amount of research projects is launched each year on national and European level.
Standardization has been identified in H2020 as one of the innovation-support measures by bridging the gap between research and the market, and helping the fast and easy transfer of research results to the European and international market, providing interoperability between new and existing products, services and processes.
The development of new and improved standards requires high quality technical information. This creates a fundamental interdependency between the standardization and research communities. Integration of European research and standardization is a recurring topic, widely recognized and promoted by European Commission (EC), European Standardization System (ESS) and other interested parties. In addition, agents of ESS and European Innovation System (EIS) have been or are being involved in projects related to that topic (e.g. FP6 -INTEREST, FP7 nanoSTAIR, BRIDGIT).
In the frame of project nanoSTAIR (GA 319092), a European strategy to support the transfer of knowledge gained through research to standards and standardization deliverables and impulse standardization in research, either at national or European level, has been proposed.
The nanoSTAIR strategic approach is based on the systematic analysis of the barriers that have been identified and of the possible ways to overcome these barriers, involving all relevant actors and identifying the possible benefits for them.
The nanoSTAIR consortium identified and selected a set of 4 strategic objectives, aimed at bridging barriers and facilitate connections between research and standardization: 1) Ensure that nanotechnology standardization is visualized as a demand to be satisfied by researchers, research organizations and research projects, 2) Provide support to research projects, from the European Standardization System (ESS), 3) Provide support to ESS, from the research community and 4) Establish and deploy a sustainable platform of services - nanoSTAIR - to facilitate the interaction between research and standardization.
In parallel, 53 targeted strategic activities - at European level only, national level or in both areas - were proposed to deploy these four strategic objectives. By type of barrier concerned, the largest number of actions (78%) is directed towards the awareness and recognition of standardization, as a strategic pillar to establish direct connections between research and standardization. Actions to provide resources and actions to improve the standardization process represent the remaining 22%, equally distributed (11% each).
In this context, the present paper describes the European scenario on research and standardization in nanotechnology and the key players, and presents a proposal of a European strategy to impulse direct “pipelines” between research and standardization.

N° O10a-1

NANOSAFETY PLATFORM

Frédéric Amblard (Plate-forme de nanosécurité, France).

The alternative Energies and Atomic Energy Commission (CEA) is a world-recognized organization. Through its core business in the nuclear field, it has built itself a solid reputation in the field of safety. This safety culture irrigates throughout its activities and especially in the field of nanotechnologies. In order to deal with the safety of its personnel, CEA has been among the first to deploy a global and unique approach to nanosafety becoming thus naturally the leader of the European project Nanosafe2. CEA has invested recently over 17 M€ in a nanosafety platform which addresses, with a unique combination of R&D and services to the industry, through its own assets or through identified expertise, the following topics:

1. Toxicology and ecotoxicology with especially a high throughput cellular screening platform for Nanotoxicology and hazard ranking,
2. occupational safety with monitoring and and measures of nanoparticles dissemination at the work stations in the industry,
3. Safer by Design approach
4. Life cycle analysis of products,
5. Management of accidental and post accidental crisis.
6. Training

Finally, it is fitted with a strong nano-characterization capability and state of the art equipment.

N° O10a-2

A SOFTWARE INFRASTRUCTURE DEDICATED TO NANOSAFETY

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The increasing fabrication and use of nanomaterials in our daily life for their unique properties bring new challenges in nanoparticles metrology. Since 2010, LNE has developed the so-called CARMEN nano-characterization platform dedicated to help manufacturers and laboratories to meet the current and future governmental regulations. Such platform aims to establish traceability routes, define measurement protocols and sampling methods. It is composed of a set of tools dedicated to physical-chemical characterization of key parameters such as nanoparticles shape, distribution, size, chemical composition, clustering state, surface chemistry…
Using the correct fleet of metrology equipments is the pedestal for the industry to follow the international agencies and policy makers. However, a key additional parameter must be taken into account in order to store, retrieve, transmit and manipulate metrology data for a safe industrial process control and short R&D cycle time. It is so-called Heterogeneous Data Management (HDM) through a dedicated software infrastructure which can be implemented in any type of industries and academic laboratories which produce or integrated nanomaterials.
In this paper, we will describe the HDM software infrastructure principle and show examples of applications on specific nanoparticles measurement applications. We will show heterogeneous data management coming from AFM and SEM equipments and compare conventional data analysis to HDM approach. A first potential industrial implementation will be presented including ISO regulations, technical specifications and/or (eco)-toxicology specifications.

N° O10a-3

MINI PARTICULE SAMPLER FOR NANOSAFETY

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ECOMESURE is an acknowledged expert in air quality instrumentation and metrology. Since 1993, we support our customers' research and application of thorough and innovative solutions for the analysis of contaminants and particulate pollutants - liquids or gases -, while respecting international regulations and taking into account growing concerns of environment and health issues. In a constant pursuit of innovation, the products are internationally sourced, but also directly developed by our team, in collaboration with our technological partners.
ECOMESURE launchs a new product for the nanoparticules research: the MPS (Mini Particule Sampler). This instrument was developed by INERIS in order to offer a portable, low-cost and simple device for particle collection and analysis. A new approach consists in using a TEM (Transmission Electron Microscopy) grid as a filter to collect particles with subsequent identification and chemical analysis.

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N° O10a-4

MINIATURE NANOPARTICLE SENSORS 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 particle surface area is of particular interest, as it has been shown in many toxicological studies to be more health relevant than traditional metrics such as particle mass or particle number.

We have constructed a miniature instrument (handheld) to measure LDSA which can serve as a nanoparticle dosimeter, the naneos partector. 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, and, in its newest version, an internal electrometer gain verification is also possible.

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.

We will present the latest developments in our instruments, and show some sample results obtained with them.

N° O10a-5

NANOBADGE

Raphaël de Thoury (NanoBadge, Alcen)

The objective of sampling device NANOBADGE and associated analysis methods is to measure engineered nanoparticles exposure in separating them from the particulate matter background.
It is used to quantify occupational exposure or characterize workplace air. The lower limits of detection are lower than 0,1 µg elemental mass /m^3 and 0,1 particles of interest /cm^3.
The collection is made in a cassette positioned on a self-sufficient and compact sampler that can be worn by operators or placed at a fix position.
Thereafter the cassette is extracted from the sampler and shipped to NANOINSPECT for analysis.
NANOINSPECT develops this service and associated products under established collaboration with Nanosafety Platform of CEA Grenoble.

N° O10a-6

NANO-SCALE OPTICAL AND HYPERSPECTRAL MICROSCOPY

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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 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. 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. 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.

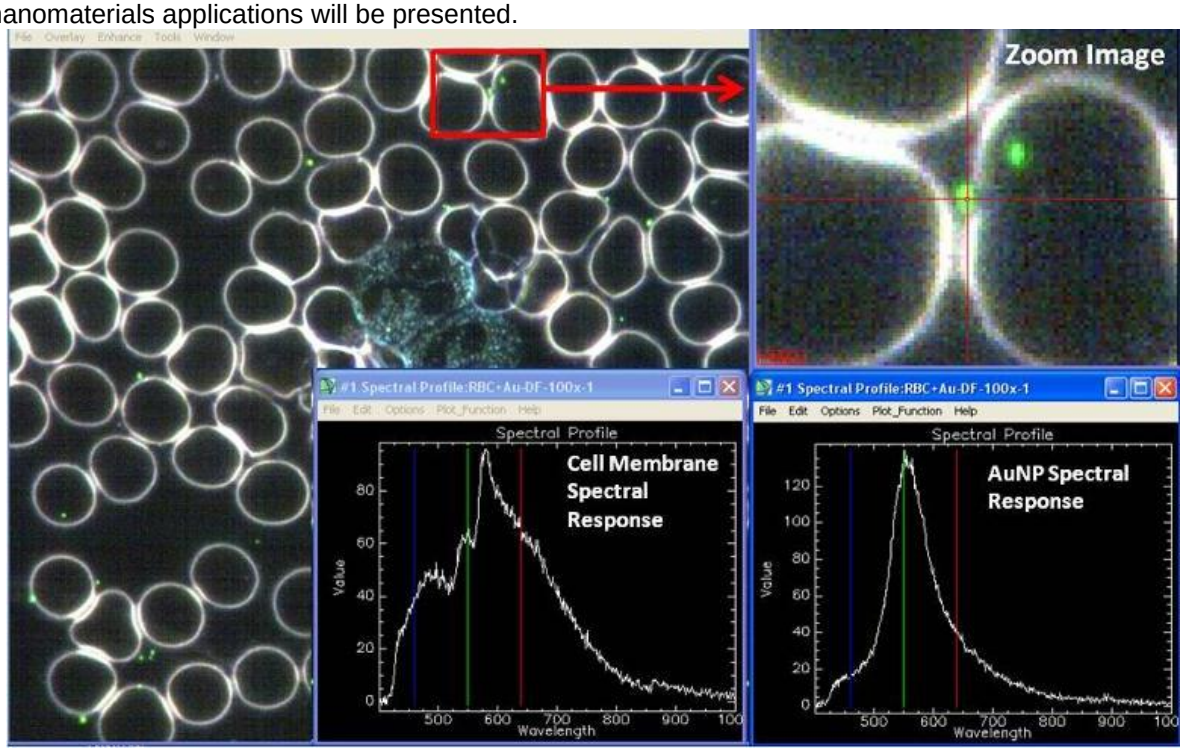

Figure 1. 50nm AuNPs (green) in Whole Blood Sample. Inset: Spectral Signature of Live RBCs (left) and AuNPs (right)

N° PL11

RISK MANAGEMENT FOR NANOMATERIALS: WHAT ARE THE EXISTING METHODS AND TOOLS?

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Nanotechnologies have been predicted to have giant market potential and this prediction is quickly becoming a reality. By 2020 it is expected that nearly every area of industry will be affected by nanotechnologies. In that context proper risk management to support safe and sustainable development, trade and use of nano-objects is a must. However, the implementation of traditional approaches for risk management cannot be successful because of several factors, in particular: a) the lack of knowledge and the uncertainties related to the effects of the nano-objects on health and the environment, b) the perception of the various stakeholders, in particular the public which sometimes compare the situation to asbestos or GMOs, c) and the lack of practical experience for the implementation of current regulations and standards adopted in various countries.

In order to overcome these difficulties it is of utmost importance to invest in research to gain knowledge on the properties and behaviours of nano-objects and thus reduce the uncertainties and answer the concerns of the stakeholders including the general public and consumers, regulatory bodies and public authorities, industry, insurance companies. Despite the very positive impact of the coordination of research with the EU NanoSafety Cluster, it will need probably several years to gain the knowledge and have it implemented in standard and regulation… and there are some questions that need to be solved now.

The iNTeg-Risk FP7 project has developed and made available a pre-standard that provides a well-structured method and describes practical tools supporting risk management related to emerging technologies (see Fig.1). The CEN Workshop Agreement CWA 16649:2013 'Managing emerging technology-related risks' adopted on June 26, 2013 combines the concept and methods from a) the ISO 31000 standard '"Risk management", that is a management standard dedicated to risk in general, and b) the risk governance framework proposed by the International Risk Governance Council (IRGC) that emphasizes the importance of communication with the stakeholders during the whole risk management process. The Emerging Risk Management Framework from iNTeg-Risk provides guidance at each steps of the risk management process from the emergence of the risk, the risk appraisal and the risk assessment up to the definition of possible risk reduction measures.

Combining the results of the projects performed in connection with the NanoSafety Cluster and the full implementation of the ERMF framework for the risks related to nano-objects would help to quicker identifying the available knowledge and the needs for further research, the need to share experiences on the implementation of regulations and standards, and the needs for communication addressing the concerns of the stakeholders. Large and integrating projects such as NanoReg addressing regulations (definition, reference methods, classifications…) should carefully pay attention to the global approach described in the ERMF and evaluate if some tools might be directly implemented for risk management of nano-objects.

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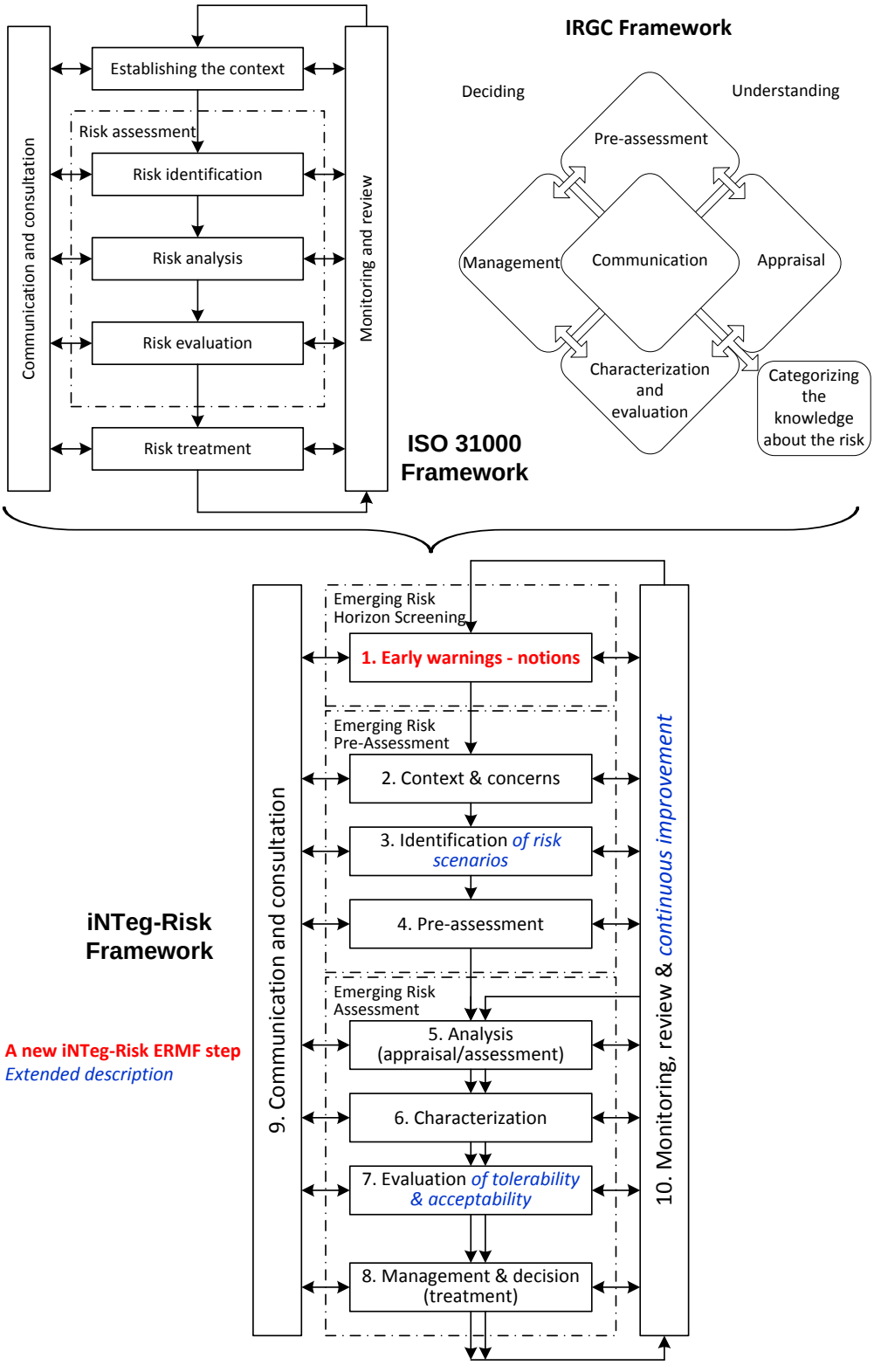

Fig. 1 : iNTeg-Risk Emerging Risk Management Framework (in CEN CWA 16649:2013 Managing emerging technology-related risks)

N° O11a-1

APPLICATION OF RISK ASSESSMENT APPROACHES ON PILOT SCALE PROCESS LINES USING NANOMATERIALS WITHIN THE SANOWORK PROJECT

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Nanomaterials are increasingly used in industrial products and cover a wide range of applications in cosmetics, functional materials, packaging and promising applications like the drug delivery which is still under investigation and development. The nanomaterials may exhibit interesting properties when compared to the same material in the micro scale. On the contrary their hazard potential may also be dramatically changed due to larger surface area and/or modified surface reactivity. Moreover, the nanomaterials due to their small size make the human beings more vulnerable to their exposure and can be transported deeper in the human body than microparticles.
The production and handling of engineered nanomaterials in the work place may pose risks of exposure and hazards to workers. This may occur during the reception, storage, production and operations like cleaning of the work places and the equipment and also during the waste disposal. So, in an industrial context, particular attention has to be done on occupational safety issues related to nanomaterials. But, no consensual occupational exposure limits (OELs) are nowadays available for nanomaterials. In this context, a series of control banding approaches specific to nanomaterials were developed in the last decade to manage toxicological risks in workplaces involving nanomaterials. These CB methods for NMs focused on inhalation risks since it can considered to be most critical route of exposure than the ingestion and the dermal route.
The present work concerns the project SANOWORK (7^{th} European Framework Program, GA: 280716), whose main objective is to develop and implement design option based remediation strategies that will prevent workers exposure and/or potential hazards. The present contribution aims to present the work realized by INERIS to demonstrate the pertinence of qualitative and semi-quantitative approaches for the assessment of occupational risks. A series of pilot scale process lines were thus investigated by INERIS in the frame to evaluate the available tools in the assessment of occupational risks. The results showed that the application of existing CB tools to the SANOWORK process lines allowed to highlighting their capabilities and limitations for the design of safe industrial process lines involving nanomaterials.

N° O11a-2

STRATEGIES, METHODS AND TOOLS FOR MANAGING NANO-RISKS IN CONSTRUCTION

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This paper presents an overview of the work carried out by the European project SCAFFOLD (GA 280535), during its 30 months of life. The aim of project SCAFFOLD is to develop, test, validate in real conditions and disseminate a new holistic, consistent and cost effective Risk Management Model (RMM) to manage occupational exposure to manufactured nanomaterials (MNMs) in construction. This will be done by integration of a set of innovative strategies, methods and tools developed by the project into consistent state-of-the-art safety management systems.

The general framework of the research conducted by SCAFFOLD considers 5 types of ***nanomaterials*** (TiO_2, SiO_2, carbon nanofibres, cellulose nanofibers and nanoclays), 6 construction applications (Depollutant mortars, self-compacting concretes, coatings, self-cleaning coatings, fire resistant panels and insulation materials) and 26 exposure scenarios, including lab, pilot and industrial scales. These scenarios represent a sample of stages of the life cycle of nanomaterials and applications select by the project (e.g. manufacturing, machining, maintenance, demolition, etc.), and operation modes of the construction processes (e.g. normal operation, maintenance, emergency fire).

The paper maps processes and exposure scenarios in construction and summarizes the results obtained so far by SCAFFOLD in the research areas of: 1) Prevention of risks in the design of new materials and processes, 2) Qualitative and quantitative assessment of occupational exposure (environmental and dermal), including measurement and modeling, 3) Collective and personal protection of workers and finally, 4) Management of nano-risks in construction companies.

Finally the document describes the structure, content and operation modes of the Risk Management Toolkit (RMT) developed by the project to facilitate the implementation of “nano-management” in construction companies. The RMT integrates the above results under an umbrella OHSAS 18001 - ISO 31000 – ISO/WD 45001. The tool is currently being validated on 5 industrial case studies and will be available for free at the end of the project (April 2015).

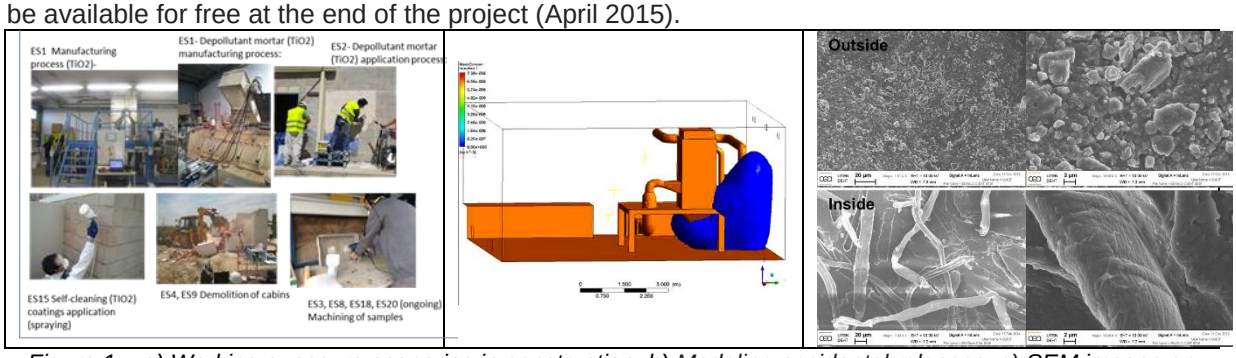

Figure 1 – a) Working exposure scenarios in construction; b) Modeling accidental releases, c) SEM images on gloves, masks and clothing involved in real scenarios

N° O11a-3

NANOMATERIALS AT THE CONSTRUCTION SECTOR – TOOLS AND GUIDELINES FOR OCCUPATIONAL HEALTH CARE UNITS

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Nanomaterials are increasingly used in the construction products, e.g. cement, wet mortar and concrete, paints, coatings, insulation materials. This is causing the fact that workers may be exposed to nanomaterials when handling these products. The most common route of exposure to nanoparticles is inhalation and the most common nanoparticle sources are dust and aerosol generating activities in the construction industry.
It is assumed that the majority of nanomaterials used are most likely safe, at least at concentrations for which workers are exposed. However, some indications of hazardous effects have been observed in laboratory studies, e.g. inflammation of the lung. Also, some nanomaterials may affect the cardiovascular system. On the other hand, workers are also exposed to other dusts and substances causing adverse health effects at the construction site.
During the Scaffold project (EU FP7) we have produced a guidance document for occupational health care units for monitoring the exposure to nanomaterials at the construction sites. The document contain recommendations for identify*ing the used products and tasks, conducting risk assessment and management. We recommend that* construction workers should have periodic health examinations (normal medical surveillance for construction workers). For workers very likely to be exposed to nanomaterials, a follow-up needs to focus on respiratory (and cardiovascular) systems.
We have also made some tests of the usability of the Stoffenmanager and Stoffenmanager nano 1.0 control banding softwares aimed for risk assessment and management purposes. The tests were performed in actual nano-coating applications. Possible exposure to nanoparticles during the application process was also examined. The exposure to nanoparticels were low during the spray application of tested polymer-based nano-coatings as indicated by the Stoffenmanager nano 1.0 software. However, both nano-coating preparations contain sensitizing compounds other than nanomaterials. Therefore, it is important to evaluate exposures to all relevant compounds of the nanomaterial preparations.
We also recommend to establish a nanoexposure registry. The register should include information of company, product, activities, probably exposed workers and exposure assessment. This nanoexposure register also provides an opportunity to assess the level of exposure and determine potential exposure - disease (health effect) association in the future.

N° O11a-4

RISK ASSESSMENT OF NANOCARBONS: USE THE ANALYTICAL HIERACHY PROCESS AND CONTROL BANDING APROACH ON SAFETY MANAGEMENT OF CARBON NANOMATERIALS

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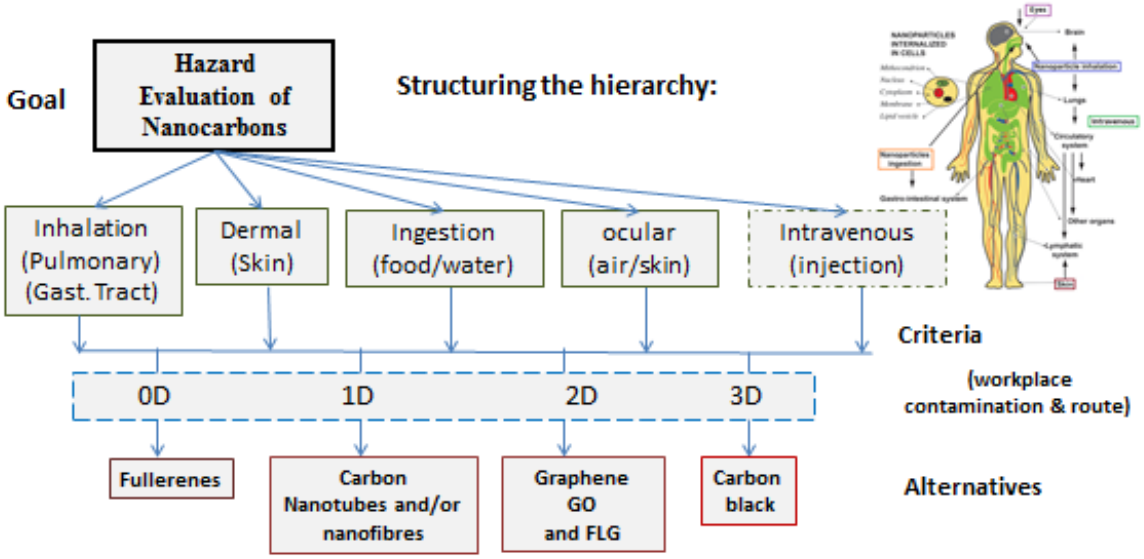
Nowadays the use of nanomaterials is increasing as new properties, functionalities, synthesis and production methods are discovered and scale up. Different uses and application of nanomaterials from automobiles industry to food packing are showing up on market. The concern about safety issues of nanoparticles has been growing in the last decade. Uncertainties, few toxicological data and reliable occupational exposure limits (permissible or recommended) combined with the increasing amount of new chemicals and materials on nanoscale level demand new approaches, specially regarding safety and environmental risk management. Quantitative and qualitative tools to manage risks are essential to provide a better safe workplace. In this work, a multicrieria method (AHP – Analytical Hierarch Process) was used in order to evaluate risks from nanocarbons materials. An integration of the AHP method with a control banding matrix was developed in order to help the risk management on workplaces. The figure presented bellow shows part of this conceptual multicriteria model.



AHP pairwise matrix

	Inhalation	Dermal (Skin)	Ingestion	Ocular (eyes)	Intravenous	(%)
Inhalation	1	4	7	6	9	52.7
Dermal	1/4	1	7	5	8	25.5
Ingestion	1/7	1/7	1	1/3	9	8.1
Ocular	1/6	1/5	3	1	9	11.5
Intravenous	1/9	1/8	1/9	1/9	1	2.2

Structured model (partial view example) using AHP (Analytical Hierarchy Process) on risk assessment of nanocarbon materials.

N° O11a-5

FIELD CAMPAINS OF MEASUREMENT OF NANOAEROSOLS : FROM SYNTHESIS OF THE RESULTS TO AN EHS PREVENTION TOOL

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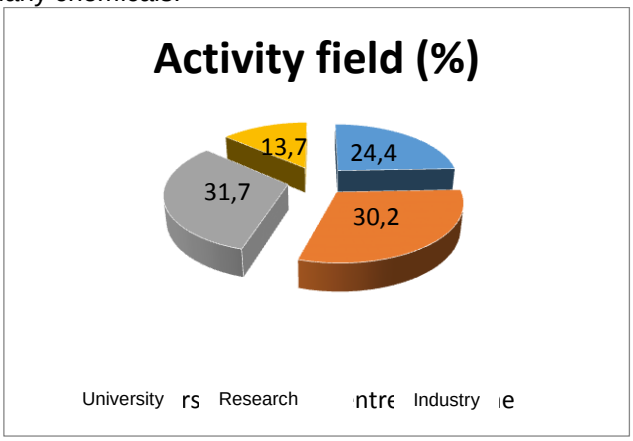
The CEA - NanoSafety Platform is developing various research topics for health and safety, environment and nanoparticles exposure, in professional activities. Numerous campaigns of measurement and characterizations of nano-aerosols have been done since 2009, in field conditions, and are still on going. About 250 scenarios of potential exposure have been studied at this time, in various fields and with many chemicals:



For each of those scenario, both characterization of the aerosols and conditions of work have been established. Characterizations are in accordance with the French guide "recommendations for characterizing potential emissions and exposure to aerosols released from nanomaterials in workplace operations". They include concentration of particles, either background or activity, size distribution, state of agglomeration / aggregation, specific surface area, shape and chemical composition.

For conditions of work, all the elements in conjunction with exposure assessment have been noted (type and quantity of materials, frequency and duration of the activity, collective protection means, individual protection ones, ….). In addition, various EHS recommendations had been proposed to join best occupational practices.

An analysis and a synthesis of the amount of data finally accumulated, first shown interesting results. Relevant points from this analysis are proposed to be exposed.

As a second part of the presentation, prevention sheets, built as operational tools for EHS manager, will be submitted to discussion. The aim is to give some global results and prevention concepts to be followed, by type of operations (weighting cleaning, …..): emissivity level as measured, influences on emission, major recommendations already done in this type of case, level of representation of the sheet,

N° O11a-6

A STANDARDIZED NON-INSTRUMENTAL METHOD FOR TRACKING WORKSTATIONS CONCERNED WITH EXPOSURE TO NANO-OBJECTS AND THEIR AGGREGATES AND AGGLOMERATES IN COMPANIES DEALING WITH ENGINEERING NANOMATERIALS

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The French national epidemiological surveillance program EpiNano aims at surveying mid- and long-term health effects possibly related with occupational exposure to either carbon nanotubes (NTC) or titanium dioxide nanoparticles (TiO2). EpiNano is limited to workers potentially exposed to these nanomaterials (NM) including their aggregates and agglomerates. In order to identify those workers during the in-field industrial hygiene (IH) visits, a standardized non-instrumental method is necessary especially for epidemiologists and occupational physicians unfamiliar with ENM exposure metrology.
A working group, Quintet ExpoNano, including national experts in NM metrology and occupational hygiene reviewed available methods, resources and their practice in order to develop a standardized tool for conducting company IH visits and collecting necessary information. This tool, entitled "Field technical logbook", includes 3 parts: company activity, workplace, and workstation allowing a detailed description of each task, process and exposure surrounding conditions. This logbook is intended to be completed during the company IH visit. Each visit is conducted jointly by an occupational hygienist and an epidemiologist of the program and lasts one or two days depending on the company size. When all collected information is computerized in a friendly-using database it is easy to classify workstations with respect to their potential direct and/or indirect exposure. Workers appointed to workstations classified as concerned with exposure are considered as eligible for EpiNano program and invited to participate.
Since January 2014, the Field technical logbook has been used in ten company visits. The companies visited were mostly involved in research and development. A total of 53 workstations with potential exposure were pre-selected and observed: 5 with TiO2, 16 with single-walled NTC, 27 multiwalled NTC. Among the tasks observed there were: ENM analysis (8), weighing (7), synthesis (6), functionalization (5), and transfer (5). The manipulated quantities were usually very small. After analysis of the data gathered in logbooks, 30 workstations have been classified as concerned with exposure to NTC or TiO2. Additional tool validity as well as inter-and intra-evaluator reproducibility studies are ongoing. These first results are promising.

N° O11a-7

RISK ASSESSMENT IN A RESEARCH LABORATORY DURING SOL-GEL SYNTHESIS OF NANO-TiO_2

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The use of nano-TiO_2 is increasing, especially due to its characteristics as a photocatalytic agent in self-cleaning surfaces, sun-screen barrier in cosmetics among other uses.

In general, the in-vitro and in-vivo tests carried out with micronized and nano-sized titanium dioxide have demonstrated a potential for harmful health effects in humans and, according to NIOSH, exposure to ultrafine TiO_2 should be considered a potential occupational carcinogen agent, resulting from a secondary genotoxicity mechanism related to particle size and surface area.

The occupational safety and hygiene (OSH) issues in the nanotechnology research laboratories get particular attention resulting from the increasing activity.

Control banding (CB) based risk assessment tools for nanoparticles are discussed as useful tools for risk assessment related to worker's exposure to manufactured nano-objects, agglomerates, and aggregates (NOAA). At the same time several direct-reading equipment and sampling media to subsequent analysis are available to assess exposure, together with the first proposals of occupational limit values and provisional reference values for groups of NOAA.

The aim of this study is to evaluate the suitability of qualitative risk assessment tools to assess nanoparticles exposure risks in a research work environment during sol-gel synthesis of nano-TiO_2, and comparing these with a condensation particle counter measurement.

For exposure assessment a condensation particle counter TSI CPC 3007 was used during the process. The air was collected in the vicinity of the worker position but not in his breathing area, thus not being a personal sampling. The qualitative risk assessment was performed using the CB Nanotool 2.0 and the Stoffenmanager Nano module 1.0.

Parameter	Concentration (particles/cm^3)
Mean concentration	25 400
Minimum concentration	14 700
Maximum concentration	132 000
Mean noise (background concentration)	18 900

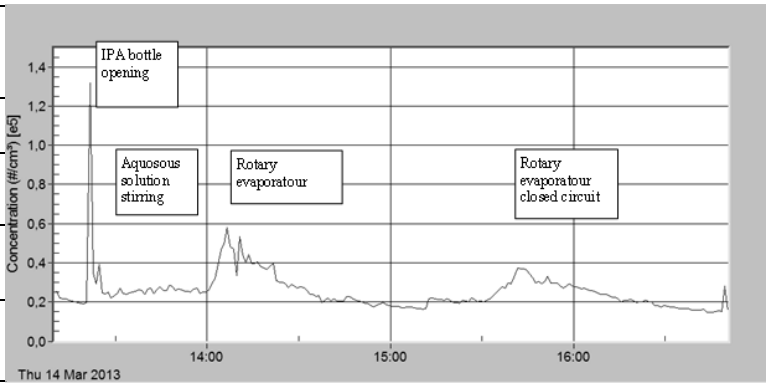

Figure 4 – Mean, minimum and maximum concentration of nanoparticles and time variation

The mean concentration obtained during the process is far below the reference value for nano-TiO_2 (40 000 particles/cm^3), raising 6 500 particles/cm^3 over the background concentration, while it is possible to identify some concentration peaks in some phases of the process.

Table 1 – CB Nanotool and Stoffenmanager Nano risk assessment results

Task	**CB Nanotool**			
	Severity band	**Probability band**	**Overall risk band**	**Control required**
Sol-gel process	Medium	Extremely Unlikely	RL1	General ventilation
	Stoffenmanager Nano			
	Hazardous class	**Exposure class time**	**Risk time**	**Exposure class task**	**Risk task**
	D	1	II	2	II

Using direct-reading CPC equipment and CB Nanotool it is concluded that the risk is low, while by using the Stofenmanager Nano the obtained results are more conservative.
It is already possible to perform risk assessment in the nanotechnology field, by using both quantitative and qualitative methodologies.

N° O11a-8

ANNOUNCEMENT ON HAZARDOUS SUBSTANCES 527 - MANUFACTURED NANOMATERIALS

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In May 2013 the „Committee on Hazardous Substances“(AGS) published the announcement on hazardous substances 527 “Manufactured Nanomaterials”. The AGS as the advising committee for the Federal Ministry of Labour and Social Affairs in Germany gives practical guidance for the assessment and management of risks during the occupational handling of MNM (Manufactured NanoMaterials). This announcement is based on the recommendation of the European Commission of 18 October 2011 on the definition of nanomaterial. There is one crucial difference. The announcement excludes natural and incidental nanomaterials.
Of particular interest the AGS considers the gathering of information to the MNM and the possible exposure. Most important for material specific data is the information of the manufacturer or distributor given in safety and technical data sheets. The material may be classified by form, size distribution, specific surface, surface modification, solubility or dustiness. Information to the possible exposure can be derived from description of the process. The announcement describes the probability of the release of MNM - decreasing from manufacture of MNM by gaseous phase synthesis or milling of the coarse material, handling of MNM powders to the machining of solid wrought material containing MNM through to the processing of MNM bond in a liquid matrix or paste.
For the risk assessment five criteria are proposed:

- Legally binding, health-based limit value
- Expert proposal for limit value or foreign limit value
- Internal action levels (based on reliable data)
- Derived No Effect Level (DNEL)
- Benchmark Level Concept of IFA

If none of the criteria is applicable or used a potency differentiating factor of 2 shall always be taken into account for biopersistent nanomaterial without specific toxicological properties and without fibrous structures. This leads to a reduction of the legally binding limit value to one half if the material is nano scaled. This assessment criterion should not be higher than 0.5 mg/m³ (at a density of 2.5 g/cm³).
The ranking for protective measures follow the STOP concept (Substitution, Technical-, Organizational and Personal protective measures).
The control of the effectiveness of protective measures might be difficult especially for low limit values using mass concentration as metric. Rather long measurement periods will be necessary. Using particle number concentration as metric could be an alternative but no generally recognized method exists yet.
Finally the announcement pointed out the importance of detailed working instructions, information and courses for the employees when nanomaterials are applied.

N° O11a-9

QUALITATIVE RISK ASSESSMENT DURING POLYMER MORTAR TEST SPECIMENS PREPARATION – METHODS COMPARISON

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Risk assessment is an important part of the process to achieve safer and healthier workplaces in the nanotechnology field. In recent years several approaches have been developed to make that possible, since the traditional risk and exposure assessment methods seem to be not fully adequate to assess risk related to nanotechnologies. The validation of the different proposed methods is a on-going work in the scientific community.
This paper presents a qualitative risk assessment comprising the comparison of different methods during the production of polymer mortars (PM).
PM with nanoalumina (45 nm) was developed as an alternative solution to improve fire behaviour of this kind of materials. Mortar formulations were prepared by mixing foundry sand with unsaturated polyester modified with nanoalumina (2,5% by weight of resin). The laboratory scale production process was divided in 3 main phases: 1- Pre-production: handling, weighing, adding to the resin and cleaning; 2- Production: stirring, pouring into the mould and cleaning; 3- Post-production: demolding, cutting and cleaning. The nanoalumina is present in powder (phase 1), suspension (phase 2), inserted in a matrix (phase 3), which allow testing the assessment methods in three different situations.
The risk assessment involved in the manufacturing process of PM was made by using the qualitative analyses based on: French Agency for Food, Environmental and Occupational Health & Safety method (ANSES); Control Banding Nanotool (CB Nanotool), Ecole Polytechnique Fédérale de Lausanne method (EPFL); Guidance working safely with nanomaterials and nanoproducts (GWSNN); Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro, Italy method (ISPESL), Precautionary Matrix for Synthetic Nanomaterials (PMSN); and Stoffenmanager Nano. Figure 1 shows the obtained results of the application of the 7 different risk assessment methods for all the considered stages.

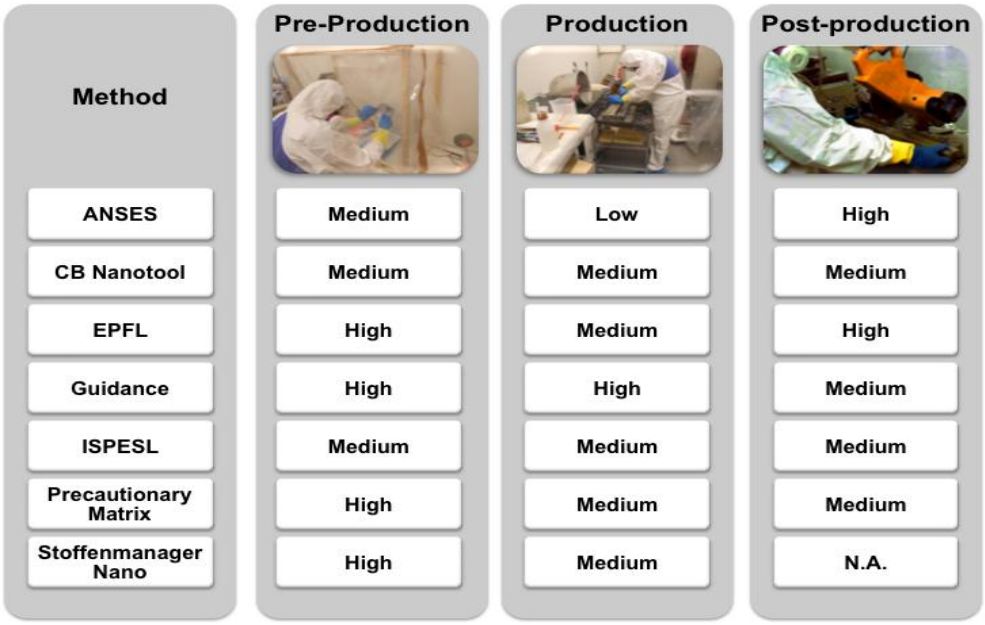

Figure 2 – Comparing the results of the different risk assessment methods
(N.A. – Non Applicable)

It was verified that the used methods produce also different final results. In phases 1 and 3 the risk assessment tends to be classified as medium-high risk, while for phase 2 the more common result is medium level. The consideration of different assumptions as risk determinants could explain some of the found differences. It is also relevant to consider the sensibility of the methods regarding the

different exposure scenarios, as some of them give the same risk level for the three different phases. It is necessary to improve the use of qualitative methods by defining narrow criteria for the methods selection for each assessed situation, bearing in mind that the uncertainties are also a relevant factor when dealing with the risk related to nanotechnologies field.

N° O12a-1

STAKEHOLDER ENGAGEMENT IN NANOTECHNOLOGIES. DIALOGUE AND OUTREACH FOR RESPONSIBLE RESEARCH & INNOVATION IN NANOTECHNOLOGIES

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Stakeholder engagement and dialogue are essential to the responsible development of nanotechnologies in Europe. For 3 years (2013-2016) NANODIODE establishes an innovative, coordinated program for outreach and dialogue throughout Europe to support the effective governance of nanotechnologies.
NanoDiode seeks to operationalize the notion of *Responsible* Research and Innovation (RRI) within nanotechnologies. The project integrates engagement activities at all levels of the R&D process: from policymaking to industrial product.

Education and dissemination activities have a limited effect if they are not consistently embedded in wider governance systems.

A concerted effort for nanotechnology governance needs to operate simultaneously at the agenda setting of R&D processes, and at the diffusion of the outcomes in society.

A strategic element is bringing together stakeholders, from policy makers to market regulators including technologists, industry, CSOs, trade unions, risk assessors, to enable a process of *RRI* to explore possibilities for synergizing regulation in nanotechnology risk assessment, while at the same time operationalizing a precautionary and yet innovative approach.

It is imperative that existing knowledge gaps are addressed to allow for a reliable risk assessment- especially relating to the hazards and exposure to toxic nanomaterial.

This calls for a paradigm change in the concept of risk assessment, putting the emphasis on an exposure- rather than a hazard-based approach, making ‘concern’ an accepted qualifier.

This relates as well to the advocated source-oriented *'safety by design'* approach for responsible nanotechnologies, including the behaviour of manufactured nanomaterials along their life cycles.

In a series of workshops starting at the NANOSAFE 14 conference stakeholders will be invited to collectively identify sensible ways forward for risk assessment and regulation for manufactured nanomaterials in Europe.

Conclusions will be drawn and when appropriate be reworked in an advice for further consideration under Horizon 2020.

The overall objectives of NANODIODE are:

- Developing new strategies for outreach and dialogue along nanotechnology value chains ;
- Organizing engagement and dialogue at the 'upstream' level of research policy ;
- Enabling processes of co-creation during research and innovation ;
- Professionalizing nanotechnology education and training ;
- Establishing a coherent program for outreach and communication on nanotechnologies ;
- Assessing the impact of the project's activities, establishing links between the various levels of governance, and providing policy feedback with a view to Horizon 2020.

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N° O12a-2

NANORESPONSIBLE DEVELOPMENT: FRAMING A MODEL OF INNOVATION MARKET UPTAKE OF NANO-ENABLED PRODUCTS

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A large part of the literature on diffusion of innovation focuses on its adoption. Very little research asks why discontinuance occurs, or why a particular innovation fails after having been adopted before. Understanding the issue of rejection of innovation in a situation of uncertainty about environmental and safety risks, can be crucial in designing future responsible and commercially successful nanotechnological innovations.
The goal of the current research is to provide a framework for future studies on determinants of market adoption of innovation in conditions of uncertainty about environmental and safety risks. Specifically, the aim is to give a framework to assess if and to what extent an innovation diffusion process is influenced by new scientific knowledge regarding risks, in a situation of uncertainty regarding these risks.
Indeed, it is suggested that consumers might be disillusioned with innovations as a consequence of new scientific knowledge about their safety (hazard) and harmful side-effects [1]. Unfavorable new scientific knowledge acts as a negative demand shock which might influence an eventual adoption of innovation (for example, through market potential). To our knowledge, despite the economic importance of this issue, the link between new scientific knowledge and adoption (rejection) of innovations is poorly documented.
After surveying different options, we discuss our proposed methodology: our approach is to identify an appropriate case study with no early concerns about health and/or environmental risks of a past innovation, but with further introduction of new scientific information about the hazard. In the given context, a case study of the chemical Bisphenol A, which has been widely used to make plastics for more than 50 years and this now being phased out in the EU and elsewhere [2], is proposed to test empirically the hypothesis about the impact of new scientific knowledge about risks on the rate of sales growth as a first step. A fundamental Bass model provides a framework for the current research [3]. While a basic diffusion model of innovation assumes a constant market potential, in practice this assumption seems unrealistic [4]. As a result an extension of the model that relaxes the mentioned assumption and that includes new scientific knowledge proxies is developed in order to test the given hypothesis. As a second step, it is suggested to adapt the model tested with Bisphenol A for nano-industry to help nanotechnology firms prevent rejection of innovation by the market and adjust their strategies accordingly. Therefore, we will work on a second case–study corresponding to an innovative nano-enabled product currently being introduced on the market, to contribute not only to better knowledge for nanoresponsible development but also to the emerging literature on the management of uncertainty.

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N° O12a-3

LICARA - GUIDELINE TOWARDS SUSTAINABLE COMPETITIVENESS OF NANOPRODUCTS

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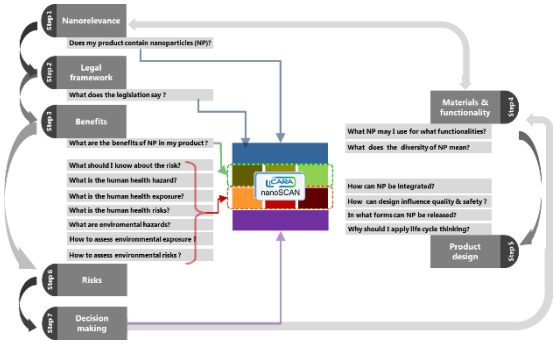
The EU FP7 project LICARA (Life Cycle Approach and human Risk Assessment, product stewardship and stakeholder risk/benefit communication of nanomaterials) has elaborated the LICARA guideline for SMEs in order to support their decision making towards developing safe and sustainable nanoproducts. SMEs - in contrast to large companies - often lack personnel resources to elaborate in-depth knowledge and to develop a product with a compelling “story”. The guideline also intends to facilitate the communication within the value chain of nanoproducts. Especially the SMEs should be supported to document their efforts for best practices and to communicate with their suppliers, clients, consumers and the authorities.
The LICARA guideline is directed to SMEs that produce nanoparticles for a wide or narrow field of applications or intermediates with nanoparticles or end products with nanoparticles and nanomaterials.
The LICARA guideline is accompanied by a prototype semi-quantitative tool, the LICARA nanoSCAN, that facilitates the implementation of the guideline.
The LICARA guideline is aimed to assess qualitatively the benefits and the risks of engineered nanoparticles, nanomaterials and nanoproducts. However, it is possible to use the guideline and the tool also for incidental nanoparticles provided that the composition and the physicochemical properties of the nanoparticles are known. The guideline is generic and therefore the user has to add sectorial knowledge about the detailed business case and the specific regulatory risk assessment for the nanomaterial or nanoproduct considered.
The first part of the LICARA guideline is structured in seven steps, raises relevant questions to be answered and provides background information. The second part describes the modular LICARA nanoSCAN, which enables you to answer relevant questions concerning your innovative material or product and evaluate the results in a semi-quantitative way. The third part presents further information for further steps and gives information on case studies.
Both the LICARA guideline and the LICARA nanoSCAN are structured in modules: the guideline in “steps” and the tool in “boxes”. The user may start with any module and select and apply only the modules of interest. Furthermore, the user may also update and re-evaluate the results from the application of the guideline and the tool (please see figure).The basis of this guideline is the scientific work of three research institutes TNO, Empa, RAS and the experiences of the private sector NCB, SNT, Freso, Nanothinx and AGPYME, which have been partners in the consortium of LICARA in the EU FP7.



Session 12
NANORESPONSIBLE DEVELOPPEMENT

N° O12a-4

THE NE^{3}LS NETWORK'S QUADRUPLE HELIX MODEL OF INNOVATION TOWARDS A RESPONSIBLE DEVELOPMENT OF NANOTECHNOLOGY.

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Many experts believe that nanotechnology innovation process will lead to important social changes. However, societies need to develop ways to take advantage of nanotechnologies' benefits while lowering the risks associated with it. As an answer to this question, many countries around the globe are investing in social and safety aspects of nanotechnology development. However, this knowledge is not integrated into a comprehensive model of innovation.
In Canada, the research community is still waiting for a national strategy on nanotechnology that will foster its development but also help in assessing crucial questions about it. At the moment, the initiative on nanotechnology regulation mainly relies in the hands of the provincial governments. The province of Quebec, was the only one in Canada to create an initiative that evaluates the important questions surrounding nanotechnologies development: the Ne^{3}LS (**N**anotechnology, **E**thics, **E**nvironment **E**conomy, **L**aw, **S**ociety issues) network concept.
From 2010 to 2014, the Ne^{3}LS network has focused on maximizing interdisciplinary research and training activities, creating networking possibilities between research communities as well as establishing links with Asia, USA, South America and Europe. The second phase of the Ne^{3}LS network's development will continue until 2021 and aims at accompanying nanotech development and assessing its impact. In addition, this initiative will also promote the quadruple helix model of innovation for responsible development of nanotechnologies. This model will make a step further than the one of Etzkowitz and Leydesdorff (2000) by including social aspects as a part of nanotech development. In order to achieve its goals the Ne^{3}LS network will interact with all actors of innovation: scientists, governments, industry representatives as well as the public. In this presentation, we will discuss about the Ne^{3}LS network theoretical and practical frameworks that will be implemented to achieve nanotech responsible development in Quebec.

N° O12a-5

SOCIO-ECONOMIC ANALYSIS OF A NANO-ENABLED TECHNOLOGY: NANO-TiO_2 COATINGS SOLAR PANEL EFFICIENCY

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While alleged properties of nanotechnologies are generally known, their development may be undermined because of scientific uncertainty surrounding their potential health and environmental impacts; Hence the ongoing efforts from international organizations (OECD, European Commission, CEN) to promote their socio-economic analyses.

But, to our knowledge, very few studies have performed case-studies of full comparison of potential social costs and alleged benefits (but see [1]).
Our work consists in such a socio-economic analysis in the specific case of a nano-TiO_2-based coating that can be sprayed onto solar panel surfaces. Relying on photocatalytic properties of TiO_2, the coating is expected to prevent the decreasing in efficiency of solar panels due to their organic fouling [2].

This study, whose perimeter is the whole 2013 French photovoltaic park, is based on the comparison of two alternative scenarios: a benchmark scenario corresponding to the actual *status quo*, namely the absence of any cleaning operation on the solar panel; and a scenario corresponding to the progressive treatment, over 5 years, of the solar panels. Because different products relying on the same technology may have different characteristics and performances two different marketed products were studied in the second scenario.
From a methodological point of view, our study relies (1) on the identification of the major potential impacts expected from the development of such a technology, (2) on their quantification, and (3) on their monetization.

Adressing the above questions, issues and related uncertainties did take the authors to consider a wide set of scientific and technical fields of expertise. The study is thus based on interviews of experts from different disciplines, namely metrology, toxicology and ecotoxicology.
The main benefit is obviously linked to the preservation of solar panel yields which allow the owners to sell electricity whereas they could have not in the benchmark scenario. Simultaneously, preserving solar panel yields prevents the emission of greenhouse gases that would have been emitted if the electricity has had to be produced by the French energy mix.

Concerning the costs – beyond the costs of implementation themselves – the potential impacts on health and environment were studied. This first required a full characterization of the aerosols sprayed on the panels and potentially released in the environment or inhalated by workers in charge of the panels' coating. No relevant ecotoxicological impact has eventually been found. Concerning health issues, chronic bronchitis and lung tumors have been identified as potential adverse effects. This issue being highly uncertain, we voluntarily developed a worst-case scenario in terms of exposure coupled with state-of-the-art toxicological results [3, 4].
Our study concludes to a positive benefit - cost index even with the most conservative assumptions corresponding to a precautionary approach.

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N° O12a-6

AREAS OF DISCUSSION ABOUT WORK, RESOURCES FOR PREVENTION OF RISKS RELATED TO NANOMATERIALS

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The emergence of nanomaterials is generally considered to be a major technological revolution in many fields. This revolution is also associated with uncertainties about the effects of these new materials on human health and the environment. To date, there have only been a few SHS studies on risk management practices, neither in manufacturing nor in handling of nanomaterials within industrial units. The effects on professional practices are also slightly considered in terms of potential risks.
This intervention is therefore part of a research program "Building and managing the risks associated with nanomaterials in industry and research laboratories", whose objective is to explore ways of managing this emerging risk. This multidisciplinary research project involves prevention specialists (chemistry, metrology) and researchers in the social sciences (sociology, ergonomics). It leverages the partnership between two different laboratories: the National Institute for Research and Safety (INRS) and the laboratory PACTE (University of Grenoble).
This paper describes the ergonomic intervention performed at a technological transfer platform that aims to promote the nanostructured composites, from the raw material to the finished product, particularly carbon nanotubes. In the context of a platform extension project, this intervention analysed workers current practices to improve the existing conditions and provide insights in terms of organization of work, planning of workspaces, formalization of procedures, etc.
The methodology used was conducted in two phases:
• A first phase of comprehensive interviews and observations of work activities. It indicated that employees encountered a shared representation of the risks associated with nanomaterials ("dust", "ultrafine particles", "facilities designed for handling nanos") but were faced in their practices with many questions about the preventive measures to be taken.
• A second phase of accompanying the platform for the construction of a preventive approach by setting up four working groups (Protection Personal Equipment (PPE), flows, waste, maintenance). It expresses how discussion areas about work allow identifications of concrete actions for risks prevention. Indeed, collective exchanges promote exploration of the causes of difficulties in the handling of nanomaterials and looking for possible improvements.
The case-by-case approach called by nanomaterials involves exchanges places to collectively build solutions and action rules adjusted to the work context. These discussion areas about work promote the production between actors of local and revisable agreements and let work, for a while (Detchessahar, 2009).

P1-1

SINGLE STEP SYNTHESIS OF GRAPHENE OXIDE USING AGRICULTURAL SUGARCANE WASTE MATERIALS

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In this work, we report a new synthetic method to produce graphene oxide (GO) using sugarcane bagasse oxidation under muffle atmosphere (SOMA-GO) method. A method has been designed and developed to catalytically convert solid agricultural waste into value added products, such as graphene oxide. We produced directly graphene oxide by using sugarcane bagasse oxidation under muffle atmosphere. Structural properties of obtained product were characterized by X-Ray Diffraction (XRD), Fourier Transform Infra Red (FT-IR) spectroscopy, Field Emission Scanning Electron Microscope (FESEM), High Resolution Transmission Electron Microscope (HRTEM) as well as Raman spectroscopy. From the XRD pattern of GO clearly demonstrate the formation of strong and sharp peak at $2\theta = 11.6$ corresponds to an interlayer distance of 0.788 nm (d_{002}) for the AB stacked GOs. The absence of any other peak of graphite confirms the complete conversion of sugarcane bagasse into GO. Further HRTEM image clearly shows the GO sheet of carbon layer structure which indicates that the GO sheet that was oxidation from sugarcane bagasse. A selected area electron diffraction (SAED) pattern of GO that was taken using a selected area from the GO sheet and the diffraction pattern image show that the resulting GO has been restored into the hexagonal graphene framework. This study has improved upon an alternative method for efficient manufacturing of GO from agricultural sugar bagasse in single step without addition of toxic chemical and less time consuming. This is the first achievement to produce graphene oxide with well graphitization using agricultural sugarcane bagasse. These waste materials could supersede the use of costly and often toxic or highly-flammable chemicals, hydrocarbon gases, carbon monoxide and hydrogen, which are commonly used as feedstocks in recent nanomanufacturing process for carbon based nanomaterials.

P1-2

FORMATION OF SILICON NANOCLUSTERS IN SIN_X FILMS AND THEIR LIGHT-EMITTING PROPERTIES UNDER THE VARIOUS CONDITIONS OF DEPOSITION AND HEAT TREATMENT

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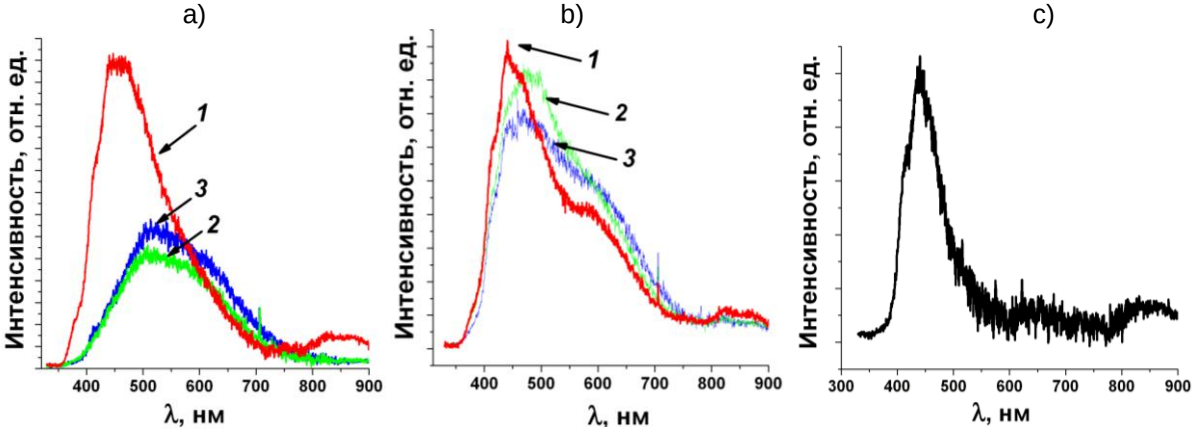
Si-rich silicon nitride films were deposited at temperature 780°C by using the low pressure chemical vapor deposition method (LPCVD). Two groups of samples were made: a group of samples with thin silicon nitride layers (not exceeding 100 nm), and a group of samples with thick silicon nitride layers (450 nm and above). After deposition of SiN_x films the stoichiometric analysis was carried out according to [1] and showed that all our series of samples were Si-rich except the one series of thin silicon nitride films (93.2 nm) where the lack of silicon (2.8-7.8%) was observed.

One part of samples was annealed in N_2 ambient at 900°C, 1000°C, or 1100°C for 60 minutes or at 1200°C for 3 minutes using the rapid thermal annealing method, another part of samples remained unannealed. The optical and structural properties of these films have been investigated by photoluminescence (PL). PL spectra of unannealed samples were structureless. Annealing of samples leads to an appearance of luminescence bands associated with defects in both N-rich and Si-rich silicon nitride films. Increasing of annealing temperature leads to redistribution of intensities between peaks and bands in the PL spectra of samples. When the annealing temperature increases, intensity of “defective” luminescence decreases. At the same time the signal at ~600 nm increases, attributed to the quantum-dimensional effect on silicon nanocrystals.

The decreasing of intensity of violet-blue PL after annealing at 1000°C can be explained by reducing of amount of nitrogen defects due to the participation of nitrogen atoms in process of nitridation (formation of additional Si-N bonds at high temperature treatment). Ammonia formed and released in process of dehydrogenation may also reduce amount of nitrogen defects in silicon nitride layers. In samples with thick silicon nitride films PL appears at annealing temperature ~1100-1200°C and above and depends on the thickness of silicon nitride layers.

References

*[1] Wilson P.R. et al., Nanoscale Research Letters, **6** (2011), 1-12.*



PL spectra of samples
a) N-rich thin films after annealing for 60 minutes, 1 – 1 – 900°C; 2 – 1000°C; 3 – 1100°C;
b) S-rich thin films (98.6 nm, 2.4-7.2% of excess silicon content) after annealing for 60 minutes: 1 – 900°C; 2 – 1000°C; 3 – 1100°C;
c) S-rich thick silicon nitride film (950 nm, 7.2-22.1% of excess silicon content) after annealing for 3 minutes at 1200°C

P1-3

NANO-ENCAPSULATION OF SHORT PEPTIDES USING ELECTROSPRAYING TECHNIQUES

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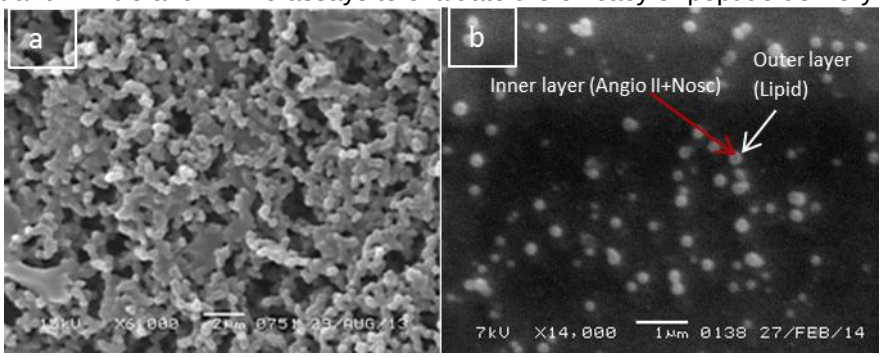
Peptides are increasingly used as medicines but their delivery to target cells and stability are posing difficulties. To circumvent these problems, encapsulation of peptides and proteins has become the focus of an alternative approach for developing novel drug delivery systems; generating nano-scale particles whose properties can be optimised depending on the desired mode of administration and target tissue. Commonly used methods for protein/drug encapsulation include solvent evaporation, spray drying, emulsification or coacervation, however these methods expose peptides to various factors that can affect their stability such as organic solvents or high temperature; also, polymer degradation may promote deactivation during these processes.
Electrospraying (ES) systems (single or coaxial) offer an alternative to overcome most of these limitations with a great potential for controlling the generation of nano-scale morphologies and optimize functionality. Angiotensin II was employed as a model in order to evaluate the stability and degradation in high-voltage electric field. Both ELISA and HPLC analysis showed that angiotensin II stability was significantly affected only when very high voltage (30kV) and low flow rates (5µL) were applied ($p=0.0015$). For an applied voltage of 30 kV, it was found that there was no significant degradation when a higher flow rate was used. ES single needle process was therefore employed to generate nanoparticles containing the Angiotensin II and modified chitosan. Nanoparticles were analysed using Dynamic Light Scattering, Nanoparticle Tracking Analysis and Scanning Electron Microscopy and shown as spherical with an average size of 60 nm (a). In a further development a coaxial system has been employed, where the inner layer (core) contained peptide and modified polymer surrounded by a lipidic shell (outer layer) with an average size of resulting NP between 100-200nm. This nano-construct method allows for a more efficient encapsulation and increases the stability of the peptide drug, while controlling its release (b). Also concentrating nanoparticles using validated filtration method followed by HPLC indicated 95% encapsulation efficacy and Transmission Electron Microscopy confirmed the multilayer encapsulation. Further work involve, release profile, cytotoxicity test and in vitro and in vivo assays to evaluate the efficacy of peptide delivery to the brain.



SEM images of: a) Angiotensin II loaded NOSC nanoparticles formed (ES single needle), and b) lipid shell nanocapsules loaded with Angiotensin II + modified polymer (ES coaxial)

P2-1

MATPUF: A JOB-EXPOSURE MATRIX TO UNINTENTIONAL NANOSCALE PARTICLES

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Aims: In recent years, the production and utilization of manufactured nanomaterials have increased which have raised concern about their potential effects on humans' health. However, the engineered nanoparticles concern very few activities at the workplace unlike the unintentional nanoscale particles (UNP). UNP are generated from usual work processes (combustion, abrasion, welding…) and thus are more prevalent and exist since a long time than the engineered ones. However, they present the same properties than the engineered nanoparticles. We propose to describe the building methodology of a UNP job-exposure matrix (JEM), a useful tool to estimate prevalence of exposure to UNP at the workplace.

Methods: A JEM is a table with in lines, job information and in columns, semi-quantitative exposure parameters like probability, intensity and frequency. Regarding MatPUF JEM, the exposure was assessed through occupations. First, work-processes generating UNP and their associated chemical families have been identified through an extensive literature review and the knowledge of an expert's panel. These work processes were related to occupations extracted from the ISCO classification (Industrial Standard Classification Occupation, edition 1968). For each combination (work process in an occupation), probability and frequency of exposure were assessed. Probability was the proportion of exposed workers in each considered occupation (0 %, < 10 %, 10 – 50 %, > 50 %) and frequency, the proportion of exposed work-time (0 %, < 5 %, 5 – 30 %, 31 – 70 %, > 70 %) during the implementation of the work process in the considered occupation. Due to the few available measurement data, intensity of exposure was not assessed. When more than one work process was associated with an occupation, the final exposure assessment was obtained by keeping the higher probability and frequency. Exposure of some occupations could vary according to industries when the involved work processes existed only in specific industries. Occupation exposure could also change over times taking account evolution of use of work processes.

Results: Over 50 processes generating UNP have been identified and classified in 9 major groups: Fragmentation of matter, Combustion, Forming and shaping, Machining, Surface treatment, Surface coating, Welding and thermal cutting, Thermal and electric engines and Other. These processes were related with seven UNP chemical families: Metal, Mineral, Carbon, Wood, Polymer (mainly plastic), Polycyclic Aromatic Hydrocarbon (PAH) and Alimentary organic (e.g. cereals). Regarding exposure results of the 1 503 ISCO occupations, around 800 were concerned by at least one work process and then exposed. Among exposed occupations, 10% were possibly or probably exposed and 30% were certainly exposed. The majority of occupations were exposed to carbonaceous, PAHs and metallic UNP.

Conclusions: Exposure results suggest that occupational exposure to UNP might be important at the workplace and concern a wide variety of workers. In order to assess intensity of exposure, we are planning measurement campaigns in French and Canadian workplaces.

P2-2

PROPOSED STRUCTURE FOR INFORMATION RECORDING OF ANALYTICAL ELECTRON MICROSCOPY ANALYSIS FOR A NANO EXPOSURE AND CONTEXTUAL INFORMATION DATABASE

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A working group of PEROSH institutes (Partnership for European Research in Occupational Safety and Health) are leading the development of a database called NECID (Nano Exposure and Contextual Information Database). NECID is a nanomaterial specific exposure database which permits the systematic and uniform gathering and documentation of data on operating conditions and measurement data of individual instruments and methods to enable a harmonized nanomaterial exposure assessment.
An important part of this database is the reporting of the analytical electron microscopy (AEM) analysis of field air samples taken alongside real time monitors. AEM is a method that can unambiguously identify which types of nanoparticles are present and give information on whether the real time measurements correspond to emissions of the engineered nanoparticles of concern or are from other incidental or background sources.
The development of NECID is currently in progress. It is important is that the database can influence and help standardise the way data is collected, analysed and recorded so that meaningful comparisons between different measurement sites and scenarios can be made.
A proposed structure for information recording of AEM analysis for the NECID database will be presented. It includes a template for the reporting of the qualitative and descriptive assessment of field air samples and for the systematic recording of images and Energy Dispersive X-Ray spectra. In addition, a structure for the reporting of semi quantitative results of particle concentrations and types are suggested.
A consensus building approach is being used and the wider the international awareness and co-operation, the more useful the NECID database will be for the nanoparticle research community.

P2-3

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 nanoparticles. Amongst a working group of PEROSH institutes, IFA and TNO have developed a database structure called NECID (Nano Exposure and Contextual Information Database), which will include exposure data and contextual information. 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 variables have been 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 were composed for research institutes and have been expanded to third parties. Different user-specific rights may be awarded for the entering, reading, reporting and export of data for different users. The NECID has been developed and a first beta version of NECID has been released and is available to PEROSH partners and several external partners. A “calculation tool” is currently in development that will facilitate the statistical analysis and comparison of entered data and the reporting of the results from NECID.

P2-4

DETECTION OF CARBON NANOTUBES AND CARBON NANODISCS ON WORKPLACE SURFACES IN A SMALL-SCALE PRODUCER

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Background: The industrial use of carbon-based engineered nanomaterials e.g. carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene nanoplatelets, carbon nanodiscs and carbon nanocones is increasing globally. The health effects of many nanomaterials are today not yet fully characterised, and to handle engineered nanomaterial a high degree of control measures and personal protective equipment are required. The release of airborne nano-objects, and their aggregates and agglomerates (NOAA) during production and handling can contaminate workplace surfaces with dust, which can be resuspended resulting in secondary inhalation exposures and dermal exposures. This study aimed to survey the presence of CNTs, carbon nanodiscs and carbon nanocones as surface contamination at a small-scale producer to assess the potential for secondary inhalation exposure due to resuspension and dermal exposure.
Methods: Eighteen different surfaces (work areas, floors, handles, other surfaces) at a small-scale producer were sampled with an adhesive tape sampling method. The chosen sampling surfaces were all associated with the production and handling of CNT powder. The tape samples were analysed with scanning electron microscopy to detect the carbon-based NOAA. Air sampling with a personal impactor was also performed on a worker producing CNTs the same day as the tape samples were collected.
Results: CNTs were detected in 50% of the collected tape samples and carbon nanodiscs in 16% (Fig. 1). No carbon nanocones were detected on the samples surfaces. CNTs and carbon nanodiscs were identified at all locations in the workplace, thus increasing the risk for secondary inhalation and dermal exposure of the workers. Both airborne CNTs and carbon nanodiscs were detected in the personal impactor samples.
Conclusions: Tape sampling is a functional method for detecting surface contamination of carbon-based NOAA and for exposure control during production at potentially any workplace that produces or handles such engineered nanomaterials. With the tape method it is possible to monitor if a risk of secondary inhalation exposure or potential dermal exposure exists through resuspension of dust deposited on workplace surfaces. With the air sampling we could confirm that carbon nanodiscs were resuspended into the air at the workplace, as these were not produced nor handled during the measurement. CNTs also were detected in the air samples, but the CNTs can be derived from either resuspension or from the work tasks with CNTs that were performed during the air sampling. Tape sampling is a complementary method to air sampling and together these two methods provide a better view of the hygienic situation in workplaces where NOAA can be released into work environments.

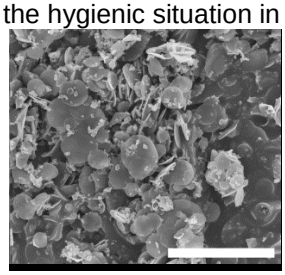
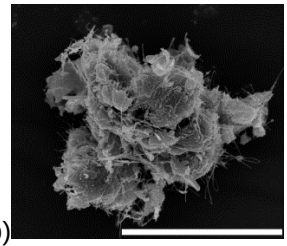
(a)  (b) 

Fig. 1. *SEM images of surface contamination of carbon-based nanomaterials from a tape sample collected nearby a saw in the production laboratory. The white bar in the images corresponds to 10 µm. a) Carbon nanodiscs. b) CNTs.*

P2-5

DEVELOPMENT OF EXPOSURE ASSESSMENT METHOD WITH THE CAMBER

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Nowadays, engineered nanomaterials (ENM) are used in various consumer products. It was reported that impacts on the health and safety depend on the use of a surface treatment and on the diameter of nanoparticles (NPs). Experiment with the titanium dioxide reports that when the surface is treated, the impacts are greater [1].In addition, the comparative study of ultrafine and fine particles shows that the same dose of ultrafine particles causes greater inflammation than fine particles. This phenomenon can be explained by the surface area of ultrafine particles is larger than the other particles [2]. However, the products release various types of NPs (shape, particle diameter and so on).Therefore, exposure assessment is difficult to define for products emitting NPs.
This study aims at developing an accurate measurement method of NPs emission (particular focusing on titanium) thanks to a chamber equipped with a HEPA filter and control the background NPs concentration by using ventilation in the chamber, then make an exposure assessment by this measurement method. So far, this measurement method was used to evaluate the quantity of volatile organic chemical substances (VOC) in daily necessities, such as formaldehyde. We performed our experiment with a spray containing titanium dioxide and tried to measure the titanium dioxide emission with this chamber method. In addition, we worked on the qualitative assessment of titanium particles. We classified titanium particles with Differential Mobility Analyzer (DMA), then used an improved electrostatic collector in order to increase the quantity of the measurement. We selected the diameter of NPs, and identified titanium with X-ray fluorescence spectrometers. We then did a morphological observation with Transmission Electron Microscope (TEM). However, it is difficult to do an accurate measurement of NPs emissions because of the airborne background. Therefore, it was necessary to control the background NPs concentration by using ventilation in the chamber, and we could establish an emission measurement of titanium and carry out a quantitative analysis thanks to ICP-MS. This method is expected to be used by workers during production process to measure exposure to NPs.
To conclude, we could grasp NPs quantitative, qualitative assessment and exposure assessment on stable background value.

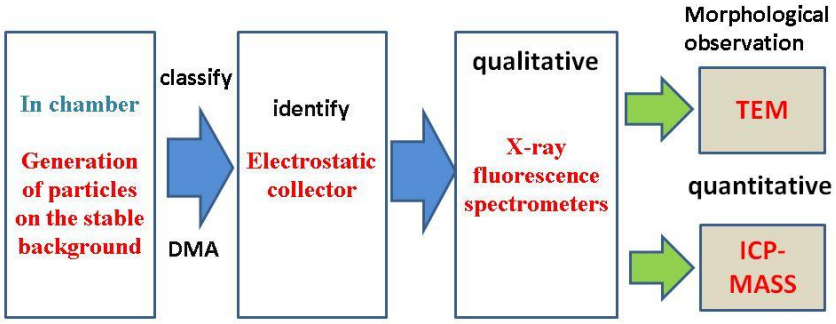

Figure 1: Protocol

Reference:

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P2-6

EVALUATION OF DUST IN THE WORKING ENVIRONMENT OF TONER HANDLING PLANTS

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Objective: Toner is a fine powder used in photocopiers and laser printers. Although the toner particle itself is a powder of micron size, nano-sized external additives attach to the surface. The objective of this study is to investigate the exposure levels and morphological characteristics of airborne dust particles in the working environment of toner handling plants, especially related to external additives attached to toner particles.
Methods: Particle concentration was measured by a pair of light scattering photometers (LD-5 for ordinary dust using infrared laser and LD-5N for nanoparticles using blue laser) whilst we observed the working operation over time at a toner handling plant. In parallel, airborne dust particles were collected on the substrates according to the sizes by means of a cascade impactor. Collected particles on the substrates were analyzed by means of a scanning electron microscope (SEM) and X-ray fluorescence (XRF).
Results: The following 4 tasks were observed: charging toner and external additives into the Henschel mixer, cleaning the Henschel mixer, charging mixed toner into the sieving machine, cleaning the sieving machine. The relationship between the tasks and particle concentrations by LD-5 and LD-5N, and the ratio of both measured values (LD-5N/LD-5) were examined. In the background, measurements of LD-5N tended to be higher than that of LD-5, and LD-5N/LD-5 was 1.6-1.7. When putting toner and external additives into the Henschel mixer, LD-5N/LD-5 rose temporarily to around 2.0 (smaller particles), and conversely, LD-5N/LD-5 decreased to 1.2-1.3 (larger particles) in other tasks. Toner particles and single or aggregated external additives were observed separately in the samples at the task charging toner and external additives into the Henschel mixer. In the samples at the task charging mixed toner into the sieving machine, most of the particles were toner particles with external additives.
Discussion and Conclusion: From measured changes in LD-5N/LD-5, it was estimated that smaller particles were observed at the task charging toner and external additives into the Henschel mixer rather than at other tasks, and the difference between suspended particles among the tasks were confirmed by the observation using SEM. LD-5N/LD-5 reflect the particle size generated at each task, which suggests that our method have relevance regarding occupational health management not only in ordinary dust but also in nanoparticles.

P2-7

EXPOSURE ASSESSMENT OF NANOPRODUCTS AND NANOCOMPOSITSUSING CHAMBER METHOD

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We have little way to solve the problem how to assess a dose of generation from consumer products including nanomaterials. It is well known that a material level of nanomaterials diffuse rapidly during bagging operation in the workplace [1]. However, there is little information of emission dose of a product level of nanomaterials such as sports gear and photocatalitic tile and a composition level of nanomaterials such as cosmetic foundation and deodorant spray.
The purpose of this study is measuring the amount of the nanoparticle generated from the product including nanomaterial by crushing and combustion or in the popular use situation. We have constructed noble exposure asssessment system (XPONA; Exposure Assessment system for Nanomaterials) using large conductive chamber [2]. Nanoparticles from the products including the nanomaterial were measured in the noble chamber we developed. The distribution was well controlled in each sampling sixteen points on a side wall of the chamber. A purification filter setted in the duct of entrance to take an indoor air to controll a background level in the chamber.
We used deodorant spray including TiO_2, kitchen antivacteria Ag spray and tennis racket string coating carbon nanomaterial and cosmetic foundation including TiO_2 and ZnO. As a result, over 1.6×10^5 number/mL particles were detected by FMPS after deodptant spray and the concentration decreased to the same concentration of chamber background in 2.5 hours (Figure 1). However, there was little particle concentration during fractured wasting. Ti was detected in each sampling filters by ICP/MS. These results indicate that Ti using for product artificially was be able to detect and separate between them and particles with other materials secondarily. We developed a new electrostatic sampling instrument for detecting in a tiny area with electron microscope, X-ray fluorescence or synchrotron micro beam.

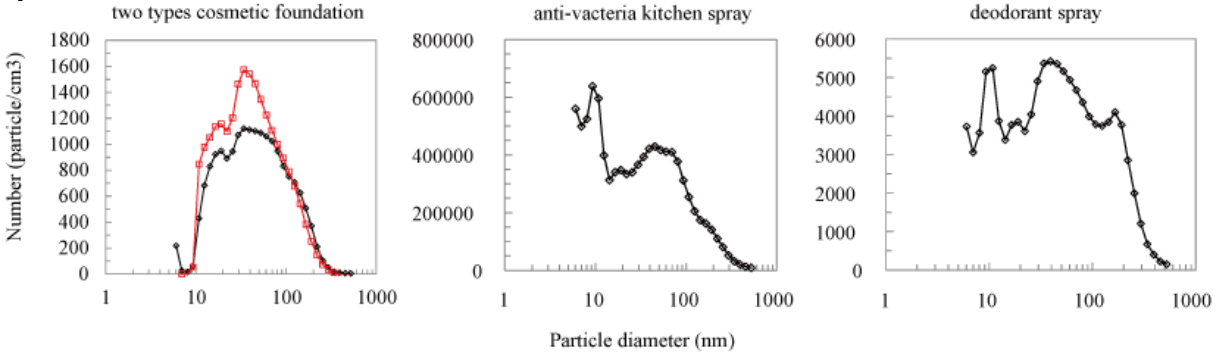

Figure 1: Particle size histogram of nanoproducts

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P2-8

EXPOSURE ASSESSMENT OF MWCNTS IN THEIR LIFE CYCLE

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Multi-walled carbon nanotubes (MWCNTs) have various beneficial characteristics and have been applied to mainly as filler in composites in order to obtain electrical conductivity or mechanical strength, etc. However, health risks from exposure to MWCNTs and fragments released from CNT-composites are concerned because of their size and shape. It is anticipated that the need for quantitative exposure assessment procedure for MWCNT.

We proposed a procedure for exposure assessment of MWCNTs[1)]. The procedure is as follows: (1) Aerosol samples are collected on five stages by a Sioutas cascade impactor (SCI), which collects size-segregated airborne particles having aerodynamic diameters < 6.6 μm, < 2.5 μm, < 1.0 μm, < 0.5 μm, and < 0.25 μm. (2) Carbonaceous substances are analyzed by a thermal-optical carbon analyser with a modified protocol (Table 1), which is originally used for environmental samples. Elemental carbons (EC) are oxidized at three steps of oven temperature (550, 700 and 920°C) in the carbon analysis. Each oxidized fraction is assigned EC1, EC2 and EC3 having different crystallinity. EC2 and EC3 in micron-sized particles are an index of MWCNTs[2)].

Exposure assessments of MWCNTs were conducted at various occupational environments in the life cycle of MWCNTs, such as production, packaging, manufacturing composites, etc. by our proposed method. The highest exposure to MWCNTs was observed near the packaging area and during furnace maintenance[2,3)]. The concentrations of MWCNTs at the personal breathing zone (PBZ) for manual and automated packaging were lower than 0.063 mg/m3 and 0.009 mg/m3, respectively[2)]. The manual packaging has been already replaced by the automated packaging. During the production of CNT-composites, neat CNTs are handled during tasks such as weighing, sonication, coating, and drying, etc. Weighing and drying showed the higher probability of exposure. The exposure of downstream users handling neat CNTs was generally lower than that of upstream users.

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Table 1 Typical protocol for carbon analysis

	Temperature（℃）	Duration (sec)	Gas
OC1	120	180	He
OC2	250	180	He
OC3	450	300	He
OC4	550	300	He
EC1	550	360	2% O_2/He
EC2	700	600	2% O_2/He
EC3	920	360	2% O_2/He

P2-9

ASSESSING OCCUPATIONAL EXPOSURE TO MULTI-WALLED CARBON NANOTUBES: AVAILABLE MEASUREMENT DATA, RECOMMENDED LIMITS AND CONTROL BANDING ANALYSES

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There is ongoing research dedicated to potential use of multi-walled carbon nanotubes (MWCNTs) in formulations for replacement of halogenated flame retardants (FRs) in selected industrial and consumer products (cables, wires, industrial fan blades, corrugated pipes, stadium chairs). This innovative application is studied as part of DEROCA FP7 project and could help reduce the amount of hazardous FRs being incorporated in composite products. However the hazard profile of MWCNTs is still not completely understood. Inhalation exposure to MWCNTs needs to be controlled considering that various studies indicated potential of airborne MWCNTs to cause harmful pulmonary effects. Content of MWCNTs in the air of various manufacturing facilities employing chemical vapour deposition or arc discharge process has been reported to be up to hundreds of $\mu g/m^3$ in the worst case scenario, thus indicating that unprotected work during production could cause adverse health effects.

Recommended occupational exposure limits (OELs) are reported by different authors and institutions and are in the range from 1-50 $\mu g/m^3$ [1-4]. OELs with metrics other than mass have also been proposed: e.g. a benchmark exposure value of 0.01 fibre/m^3 [5, 6].

Proper exposure assessment is suffering from non-existing consensus on the measurement metrics relevant to health protection, monitoring and reporting. Challenges to be resolved include MWCNTs characterisation methodology (including sampling protocols, instrument calibration, counting rules for electron microscopy) and development and application of selective, accurate and fast measurement methods for airborne MWCNTs.

In the absence of actual measurement data, risk assessors in occupational hygiene often use modelling to estimate exposure levels. In order to investigate about the potential benefits of using such approach for exposure to MWCNT, we perform sensitivity analyses of the available nano-specific control banding tools NanoSafer and Stoffenmanager-Nano. Outcomes are discussed in context of current recommended limit values and available measurement data for selected exposure scenarios within the project.

This work is funded from the Seventh Framework Programme FP7/2007-2013 under grant agreement n° 308391.

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P2-10

AN INVESTIGATION REGARDING HUMAN RESPONSES TO TONER EXPOSURE IN A TONER MANUFACTURING PLANT

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Objective: In recent years, the diameter of the toner particle has steadily gotten smaller alongside technological changes, and the 15-50nm external additive covers the surface of the toner particle. Therefore the issue of human responses to toner exposure has attracted attention. The objective of this study was to investigate the biological effects of toner exposure to the worker.
Methods: The following items were considered in advance: guarantee of voluntary participation of subjects in study, measures for securing subject privacy, methods of obtaining informed consent from subjects, notifying subjects of study results, handling biological samples collected from subjects (methods of storage and disposal, etc.), prohibiting unintended use of biological samples collected from subjects, destruction of the study data after study completion, and possible risks and disadvantages for subjects and measures for handling them when they occurred. Then, we applied for third party review of the study content, asking the Ethical Review Board of the University of Occupational and Environmental Health, Japan and obtained approval. The Subjects were comprised of 20 male workers, divided into three groups: toner-exposure group A (5 workers), toner-exposure group B (5 workers), and the control group (10 workers). Toner-exposure group A wore dust masks and did toner-handling work, toner-exposure group B wore surgical face masks and observed the work, and the control group performed routine work in the office. We conducted a self-administered survey regarding allergies before and after toner-handling work took places. The workers handled the toner for 2 hours, and we took a blood cell test (WBC, cell contents of WBC), measured biochemical indices in blood (SP-D, High-sensitive CRP, IgE) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in urine four times - before exposure, 7 hours, 20 hours, and 24 hours after the work exposure.
Results: Although the levels of WBC, neutrophils, and the 8-OHdG showed an increasing change within a normal range in the toner-exposure group A after the exposure, these changes were not significantly different vs. control. There were no significant differences in the self-administered survey between any of the exposed groups or the control group.
Conclusion: We investigated the human adverse responses of toner exposure. An adverse biological effect was not found between the toner-exposure groups and the control group. Further analysis would be still necessary to clarify the human biological responses to toner exposure

P2-11

DISPERSION STATE OF SiO_2 FOOD ADDITIVES IN GASTROINTESTINAL ENVIRONMENT

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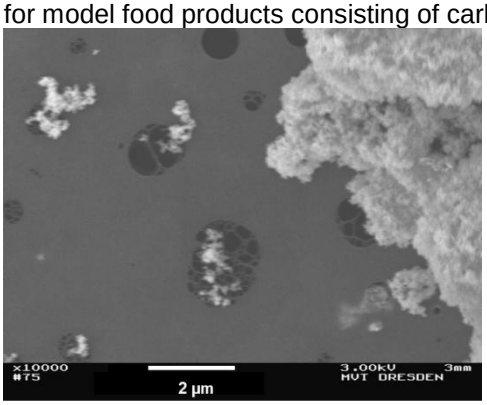
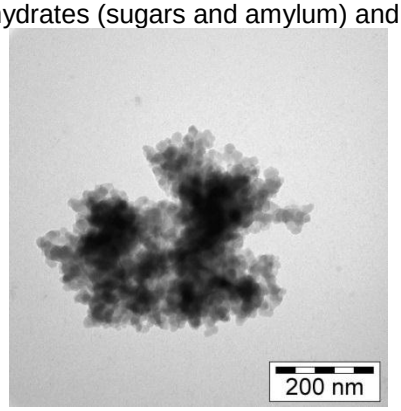
The production of nanomaterials does not only enhance the development of effective and stable products but also raises several new questions regarding potential risks to humans. One of these questions concerns the exposure of humans to nanostructured materials that are contained in various food products as performance additives.
A typical example of such additives is synthetic amorphous silica (SAS), which has been used for decades in commercial food products, e. g. as free-flow agent in soup powders (E551). SAS consists of nanosized primary particles (< 100 nm), which are fused to submicron aggregates that themselves form micrometre (or even larger) agglomerates. As to risk evaluation, it is important to understand whether structural changes may occur during food processing and – most important – after oral uptake within the gastro-intestinal tract.
The aim of this presentation is, therefore, to assess possible structural changes of nanostructured SAS when exposed to simulated human gastro-intestinal media.
In a first step, commercial SAS food powders were suspended and dispersed in physiological media with a uniform standard operating procedure (SOP). The physiological media simulated i) the acid environment of the stomach (2 hours, pH=1.3 of gastric juice) and ii) the neutral and protein-rich intestinal environment (48 hours, artificial intestinal solution – FeSSIF). Samples of defined exposure times were analysed with laser diffraction, which yields the volume weighted size distribution, and with (high resolution) transmission electron microscopy, which reveals the morphology of the SAS particles and thus allows an evaluation of possible SAS degradation (via the size distribution of the constituent particles and via internal and surface roughness). In a second step these experiments were conducted for model food products consisting of carbohydrates (sugars and amylum) and SAS additives.





SEM (right) and TEM (left) analysis for SAS particles in FeSSIF-Exposition[3]

[3] *Maier, Albers, Stintz et al. (2014)*

P3a-1

TOWARDS AN INDICATOR OF NANOMATERIAL DEPOSITION IN THE HUMAN LUNG

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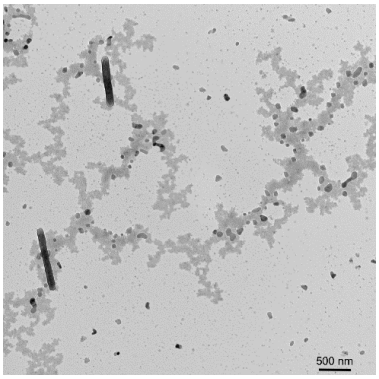
Various engineered nanomaterials (ENM) have already been incorporated to large-scale industrial processes while their toxicological profiling is still under way. In light of this fact, workplace exposure to ENM merits increased scrutinization.
Bronchial washings (BW) are diagnostic procedures during which instilled saline solution recovers the components from the epithelial surface to the larger airways of the lower respiratory system. It could also be able to provide insight to deposited particles in the lungs, i.e. the main route of human exposure to airborne particles. The aim of this work is to identify in BW an indicator of deposited engineered metal and metal oxide particles to the lungs. To this end, we have optimized the extraction of sufficiently dense nano-sized particles from BW and have subsequently performed their physicochemical characterization and semi-quantitative analysis. To this day, 60 patients have been included in the study all of whom presented symptoms of infiltrative pulmonary syndromes when admitted in Centre Hospitalier Universitaire of Saint-Etienne, France.
BW was centrifuged on top of glycerol–NaOH(aq) cushions. Most of the proteins, glycoproteins and small sub-cellular debris were thus left in the supernatant, while sufficiently dense or large particles were forced into the glycerol compartment. The pellet was then reconstituted with NaOH(aq), sonicated and measured by dynamic light scattering (DLS). The elemental compositions of both the pellet and the supernatant were measured by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES). The samples for which DLS and ICP-AES corroborated a possible particulate load were observed under transmission electron microscopy (TEM). Presented here is the nanoparticle extraction protocol, the analysis of samples from the first 9 patients and how this data could function as an alert indicator for human exposure to ENM.
This work is both original and urgent, as it is the biggest clinical study specifically designed to detect and analyze ENM in human biological samples, at a point in time when workplace exposure to ENM is expected to get higher.

Transmission electron microscopy image of nanoparticles and nanofibers deposited in the lung



P3a-2

DEVELOPMENT AND VALIDATION OF AN INHALATION SYSTEM SUITABLE FOR RODENT EXPOSURE TO NANOAEROSOLS

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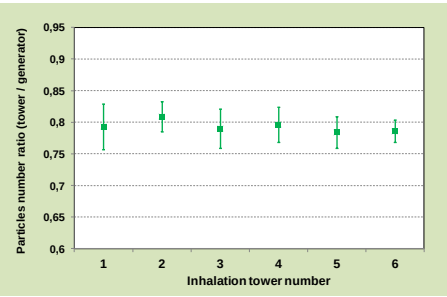
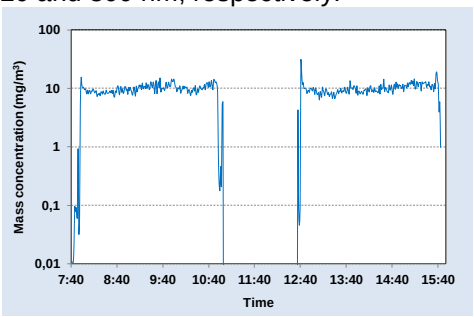
Due to the growing use of nanoparticles in industrial processes, the number of workers potentially exposed is increasing while the toxicological effects of these compounds have not been fully characterized yet. Because inhalation represents the main route of occupational exposure to airborne nanoparticles, the first organs to be exposed are those from the respiratory system. In this respect, the experimental toxicology studies conducted by inhalation in animals appear to be the most relevant for the early evaluation of the hazard associated with exposure to nanoaerosols.
In this framework, INRS has built a dedicated laboratory (inside its new nanomaterials research unit) which meets the highest requirements for animal testing in terms of protection of operators against risks associated with nanoparticles and biohazard.
The key element of this laboratory is NanoTIREx: Nanomaterial Toxicology Inhalation system for Rodent Exposure. It has been designed according to OECD guidelines for the testing of chemicals and is mainly composed of an aerosol generation system and inhalation towers for nose-only exposure. Exposure capability is around 100 rats: 50 nanomaterials exposed rats (in 6 × 9 ports manifold) and 50 control rats (in 2 × 27 ports manifold).
The integrated control of the exposure conditions (flow rates, temperature, relative humidity, relative pressure, etc) is managed and recorded using dedicated software.
The monitoring and the characterization of the aerosol are ensured by both real-time devices (condensation particle counter, optical particle sizer) and samples taken for further off-line analyses (gravimetric analysis, mass size distribution from cascade impactor, TEM observations).
This installation is currently used for 28-day inhalation studies with TiO_2 nanoparticles focusing on their respiratory and neurological toxicity as well as their toxicokinetics. As the planned test approach for these studies is risk-driven, a dry powder generation method to produce test aerosols of nanoparticles and aggregates and agglomerates (NOAA[4]) has been chosen (rotating brush generator). The NanoTIREx was tested for operation performances with target exposure mass concentration of 10 mg/m^3. As illustrated on the figures below, the validation results show that an aerosol of NOAA can be stably generated over several hours (left), the concentration being homogeneous between the different inhalation towers (right). Particle size distributions measurements demonstrated that mass- and number-median aerodynamic diameters are around 420 and 300 nm, respectively.





[4] according to ISO/TS 12901-2 (2014)

P3a-3

MEASURING AT RELEVANT CONCENTRATIONS - RADIOLABELLING AS A VERSATILE TOOL FOR SENSITIVE NANOPARTICLE DETECTION.

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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 have developed various methods of introducing radiotracers into some of the most common nanoparticles, such as Ag, carbon, Silica and TiO_2 nanoparticles. The labelling techniques are the synthesis of the nanoparticles using radioactive starting materials, the binding of the radiotracer to the nanoparticles, the activation of the nanoparticles using proton irradiation, the recoil labelling utilizing the recoil of a nuclear reaction to introduce a radiotracer into the nanoparticle, and the in-diffusion of radiotracers into the nanoparticles at elevated temperatures. Using these methods we have produced [$^{105/110m}$Ag]Ag, [$^{124/125/131}$I]CNTs, [^{48}V]TiO_2, [^{7}Be]MWCNT, [^{7}Be]SiO_2, [$^{44/45}$Ti]TiO_2, etc.. The methods are adaptable for a wide range of other nanoparticles. The so-labelled nanoparticles can be detected at minimal concentrations well in the ng/L range even with a background of the same element and without complicated sample preparations necessary.

Using our methods one can radiolabel commercial nanoparticle samples for sensitive detection in environmentally relevant trace concentrations.

P3b-1

QUANTITATIVE MEASUREMENT OF CARBON NANOTUBES RELEASED FROM THEIR COMPOSITES BY THERMAL CARBON ANALYSIS.

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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. As there are still concerns regarding the potential impact of CNTs on the health of workers and users, evaluation and control of CNT exposure are required.
Thermal carbon analysis, such as Method 5040 of the National Institute for Occupational Safety and Health (NIOSH), is often used as a quantitative measurement of CNTs [1]. This is a method to determine the fractional content of organic carbon (OC) and elemental carbon (EC). A sample collected with a quartz-fiber filter is heated in stages in a helium atmosphere to vaporize OC, after which the EC is burned by heating in stages in the presence of oxygen. The vaporized or burned carbon is completely oxidized to CO_2 with a catalyst. Then, by reducing CO_2 to CH_4 with another catalyst, CH_4 is detected using a flame-ionization detector. CNTs are detected in the EC fraction. This method is currently one of the most reliable quantitative methods for measuring CNTs; however, when a large quantity of other carbon material exists, this technique may be inapplicable.
When CNTs are used in a mixed state with a polymer as a composite material, particles of mixed CNTs and debris from the polymer itself can be released during mechanical and abrasive processing. Unattached (free) CNTs can also be released, but they may be in the minority. The harmful effects of mixed CNTs have not been fully evaluated, although Wohlleben et al. (2011) indicated that they are smaller than those of free CNTs [2]. Under the present circumstances, exposure to mixed CNTs as well as free CNTs should be measured and controlled.
In this study, we evaluated the capability of thermal carbon analysis to determine the CNT content in a mixed state with a polymer (polystyrene, PS). Samples placed in a Pt foil boat were measured by a thermal-carbon analyzer (CAA-202M-D, Sunset Laboratory Inc., USA), and the results were compared with gravimetric measurements of sample masses obtained by an ultra-microbalance. The temperature-step program was essentially based on the analytical method established by NIOSH. The optical pyrolysis correction for pyrolytically generated carbon soot from OC during analysis was not used.
First, debris from the polymer without CNTs (i.e., PS debris) was analyzed. The amount of the PS debris detected in the OC fraction was in good agreement with the gravimetrically measured mass of the PS debris, while the amount of generated carbon soot detected in the EC fraction was negligible. Next, single-wall CNT (AIST/TASC Super-Growth) powder was analyzed. The amount of detected EC was comparable to the gravimetrically measured mass of the CNT powder. Finally, debris from the 5 wt% CNT–PS composites was analyzed. The amounts of detected total carbon and EC were comparable to the gravimetrically measured masses of CNT–PS debris and the estimated CNT content (5 wt%), respectively. Further studies are necessary to assess the effect of the temperature-step program and different types of polymer.

Acknowledgment:
This is based on results obtained from a project commissioned by the New Energy and Industrial Technology Development Organization (NEDO).

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P3b-2

EVALUATION OF THE BEHAVIOUR OF SOME SULPHONYLHYDRAZONE AND *N*-ACYLHYDRAZONE DERIVATIVES AS DRUG DELIVERING SYSTEMS FOR THE TREATMENT OF DIABETES MELLITUS TYPE 2 AND CANCER

Fabio Furlan Ferreira[1], Amanda Laura Ibiapino[1], Laysa Pires de Figueiredo[1], Fanny Nascimento Costa[1], Eliezer J. Barreiro[2], Lídia Moreira Lima[2], Daniel Nascimento do Amaral[2], (1) Center of Natural and Human Sciences (CCNH), Federal University of ABC (UFABC), Av. dos Estados, 5001, Santo André, SP, 09210-580, Brazil / (2) LASSBio, Institute of Biomedical Sciences, Federal University of Rio de Janeiro (UFRJ), Av. Carlos Chagas Filho, 373, Rio de Janeiro, RJ, 21941-902, Brazil.

The search for new drugs that produce effective and specific results in the treatment of diseases such as diabetes mellitus type 2 and cancer, for example, are of great scientific and technological interest. The reduction of doses ingested by patients, fighting target tissues affected by the harmful manifestations to the organism, may provide a significant improvement in life quality of individuals as well as to reduce the side effects usually seen in some types of treatment.
Many solid pharmaceutical formulations deserve attention due to possible variations of theirs physicochemical properties, such as changes in the melting point, solubility, in true density, in the dissolution profile, among others, affecting the efficacy and bioavailability of the drug in the body. Many drugs are administered in solid form. Of these, many are polycrystalline, being easily detected by X-ray diffraction. When the crystal structures are known, the application of the Rietveld method of refinement of crystal structures is a powerful tool for the structural characterization. However, when the structure is unknown, the method may not be considered. In these cases, the first step to be taken is the efficient collection of X-ray diffraction data and the use of computational methods for the elucidation of the crystal structure for subsequent application of the Rietveld method.
In this work, we determined the crystal structures of several candidate compounds for new drugs planned in the treatment of diabetes mellitus type 2 and cancer, by using X-ray powder diffraction data and a simulated annealing approach. An important part of structural characterization is related to the analysis of the crystal habit of the crystals formed in the synthesis process. Computational methods of prediction of crystal morphology were correlated with images obtained by optical microscopy and a good agreement was found. Ongoing tests for the encapsulation of such candidate compounds to be used as nanocarriers for drug delivering systems revealed promising results and will be discussed in this work.

P3c-1

CLUB NANOMETROLOGY: A FRENCH INITIATIVE TO IMPROVE THE RELIABILITY OF MEASUREMENTS AT THE NANOSCALE.

Daniel Bernard[1], François Piquemal[2], Khalifa Aguir[3], Simone Cassette[4], Julie Carimalo[5], Yannick De Wilde[6], Sébastien Ducourtieux[2], Georges Favre[7], Nicolas Feltin[2], Brice Gautier[8], Philippe Lambert[9], Ariel Levenson[4], Guy Louarn[10], Tatiana Macé[6], Philippe Maillot[11], Jean-Marc Moschetta[6], (1)CEA – Grenoble, 17 rue des Martyrs 38000 Grenoble / France (2) LNE - Trappes, 29 avenue Roger Hennequin, 78197 Trappes Cedex / France (3) Aix-Marseille Université – IM2NP, St. Jérôme, 13397 Marseille / France (4) Thales Research & Technology France, Campus Polytechnique 1 avenue Augustin Fresnel, 91767 Palaiseau Cedex / France (5) NanoSciences France (C'Nano), CNRS/LPN, Route de Nozay, 91460 Marcoussis / France (6) Institut Langevin, ESPCI/ParisTech, 10 rue Vauquelin, 75005 Paris / France (7) LNE – Paris, 1 rue Gaston Boissier, 75724 Paris cedex 15 / France (8) INL/INSA Lyon, 7 avenue Capelle, 69621 Villeurbanne Cedex / France (9) C'Nano Grand-Est, Institut Jean Lamour, CNRS/Université de Lorraine, BP 70239, 54506 Vandoeuvre les Nancy / France (10) Institut des Matériaux Jean Rouxel – IMN, BP 32229, 2 rue de la Houssinière, 44322, Nantes Cedex 3 / France (11) STMicroelectronics, ZI de Rousset Avenue Coq, 13106 Rousset / France.

Since a decade, nanotechnologies are covering a completely new area in which manufacturers, governments and citizens have very high expectations. Indeed, nanotechnologies are offering an ever increasing number of potential applications in all the key sectors of the industry: semiconductor and micro/nanoelectronics, information and communication technologies, aeronautics and aerospace, cosmetics and foods, building industry, transport and automotive, textile …), but also in health (medication vector imaging, and therapy) and in environment (sustainable energy, water treatment). Nanomaterials, products or processes implementing them already exist, but means to measure their properties are still in their infancy. It is often difficult to develop the instrumentation capable of measuring the size, shape and physico-chemical properties of nano-objects with the required level of uncertainty. Moreover industrial processes involve quality insurance management which relies on demonstrated reliable tools for which nanometrology may have a key role.

In parallel, the development of nanomaterials raises the question of their regulation and possible risks for health, safety and environment (HSE). In this idea, a French's government decree on a compulsory annual declaration of products in the nanoparticle state issued in 2012 and took effect from January 2013. However, all reports published by government agencies and standards organizations points out the lack of tools, lack of reference materials and methodologies that would establish the traceability of measurements and thus facilitate application of the decree and comparisons. This marks a second difference compared to traditional sectors.

Support to industry in nanotechnologies and analysis of risk / benefit ratio of nanomaterials demand the development of a new metrology: the nanometrology. Moreover, there is of course no industrial federation or union specifically dedicated to nanotechnologies which now affect almost all sectors.

For these main reasons which might explain the current lack of a true industrial sector of nanotechnologies in France, LNE and C'Nano, a program initiated by the CNRS, the CEA and the Ministry of Research, created in 2011 the Club nanoMétrologie. Its objectives are:

1) to gather industrial sector, academic and government agencies to develop an inventory of instruments available in the national territory, standards and traceability chain for all the physical and chemical properties;
2) to provide metrology solutions to the needs identified by industrial in nanometrology and to the works performed by laboratories and government agencies on the toxicological and eco-toxicological properties of nanomaterials;
3) to work out and to encourage R&D programs, to support standardization and to ensure dissemination of metrological knowledge (manuals, technical procedures …).
4) to deal with very specific topics, three working groups were constituted on the themes: (a) benefits / risks of nanoparticles in health and environment, (b) traceability of measurements at the nanoscale, (c) instrumentation dedicated to nanotechnologies.

300 members are currently in the club, one third coming from SMEs, start-ups and large industrial groups (producers, companies in processing and integration, instrument manufacturers …). We will then return the main highlights of the working groups and present outlook.

Session 3
DETECTION AND CHARACTERIZATION

P3c-2

A COMBINATION OF OPTICAL AND ELECTROCHEMICAL TRANSDUCTION PRINCIPLES MERGED IN A NOVEL SENSORSYSTEM

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In recent years, nanotechnology has dynamically developed to a fundamental centerpiece in the area of science. This is due to the varied and specific properties of nanomaterials. Especially engineered nanoparticles (ENPs) show areas of interest for a wide range of applications [1]. Silver nanoparticles for example are hidden in meat packaging or mitigate body odor via the use in deodorants. The antibacterial effect of silver leads therefore to a high application in consumer products [2].

Unfortunately, the risk assessment of nanoparticles in daily products is still a very complex task and it is currently far away from being researched in depth. The question whether the benefits or the harms of ENPs outweigh is highly dependent on specific parameters like the composition, size and shape of ENPs. Thus, a precise identification and characterization is mandatory. Nevertheless, this is a challenge, since matrix components often hamper a specific detection of the analyte. For this purpose, the 7th framework programme of the EU promotes the project INSTANT (Innovative Sensor for the fast Analysis of Nanoparticles in Selected Target Products). This project will face the challenge to detect, identify and quantify important ENP properties in complex matrices (e.g. in creams and meat packages) in a single step, in order to set up a fast and cost-effective monitoring tool.

With this in mind, a sample preparation unit, two complementary label-free transduction principles and a chemometrical approach are combined. The optical sensor will provide the information about size, concentration and refractive index of the material, whereas the electrochemical part can specify its chemical composition and therefore the nature of the nanoparticle material. The optical and electrochemical part, their components and the first results measured on this combined setup will be demonstrated on a poster.

Acknowledgements: The work was performed as part of the EU-subsidized project INSTANT (FP7-NMP-2007-2013-SME5-280550). Reference nanoparticles were provided by project partner Federal Institute for Materials Research (BAM). The carbon nanotubes were kindly provided by project partner Nanordic Oy from Finland.
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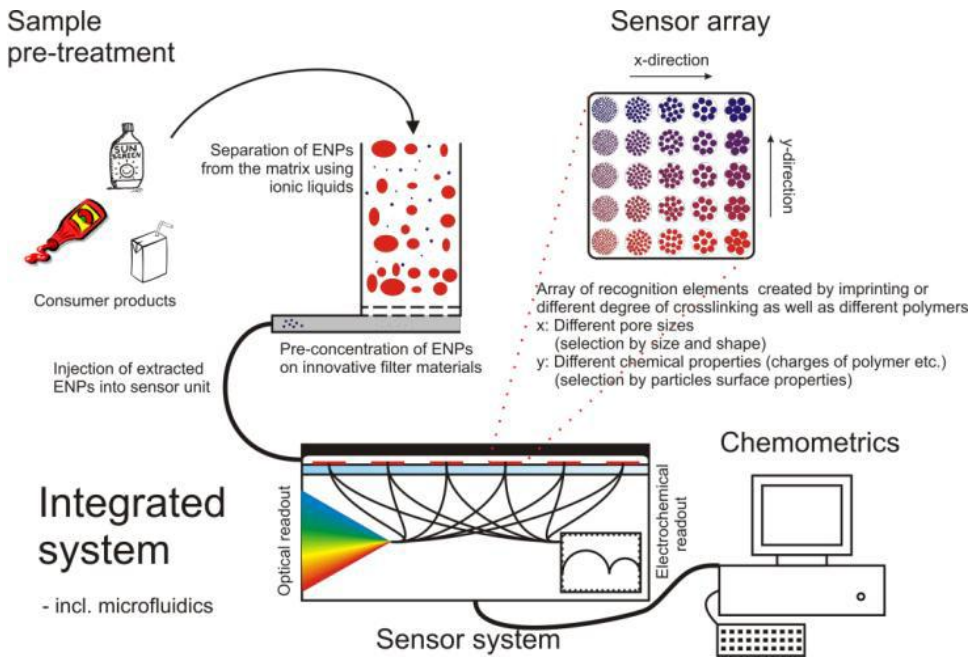

Fig.1: Illustration of integrated INSTANT device

P3c-3

PERFORMANCE ON THE VORTEX SHAKER DUSTINESS TEST METHOD AS A CONTINUOUS AEROSOL GENERATOR: TIME VARIATIONS IN PARTICLE NUMBER CONCENTRATION AND SIZE DISTRIBUTION OF AEROSOLIZED NANO-TiO_2

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Agglomerated engineered nanoparticles (ENPs) have complex shapes and exhibit a wide range of size distributions, which complicate their aerosol measurements and exposure assessments. Aerosol generators, designed on the basis of dustiness test methods that can determine the propensity of powdery materials to produce airborne dust during handling, can be helpful in the evaluation of ENP exposure potentials, and also useful in improving aerosol measurement methods.
The vortex shaker method (Maynerd et al., *J. Toxicol. Environ. Health A*, 2004; Ogura et al., *J. Phys. Conf. Ser.*, 2009) has an advantage over other dustiness test methods, in that it requires only a small volume of test material (about 1 cm^3). However, changes in ENP dustiness during agitation have not been fully evaluated; therefore, the purpose of this study is to verify the time-series behavior of ENP dustiness, using the vortex shaker method, and thereby improve the method as a polydisperse ENP aerosol generator for a significant period of time.
Several kinds of TiO_2 ENPs, with similar primary particle shapes and size (ca. 20−30 nm) were examined as test samples. The particle number concentrations and particle size distributions of generated ENPs were quantified with the use of aerosol-measuring instruments (OPC, CPC and SMPS). The results show that all the ENP samples had similar particle number concentration and size distributions, with the mode falling within the sub-micron range just after the beginning of agitation. Few nano-sized particles were generated by the method. Some samples also showed that the concentration of sub-micron sized particles quickly decreased after agitation had commenced. The time-series changes in the particle number concentrations and size distributions were different for each sample, even though the primary particle size, shape, and chemical composition were almost similar. These results suggest that the surface characteristics of the particles, e.g., the hydrophobic treatment, the TiO_2 crystal forms or the purity of the metal oxide, affect the agglomerative properties of the TiO_2 ENPs. In the case of some samples, the concentrations of generated particles decreased with time, where small stainless beads (1/16 inch in diameter) were added to prevent the formation of agglomerates and particle-deposited layers inside the glass tubes. Consequently, the particle number concentrations and size distributions of the ENPs were maintained for several hours for some of the samples.
These results indicate that the vortex shaker method is applicable in the evaluation and improvement of ENP aerosol measurements, and that the particle number concentration and size distribution of generated ENP aerosols can be controlled by the addition of small balls.

P4-1

IN VITRO EVALUATION OF NICKEL OXIDE NANOPARTICLE'S TOXICITY

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Nickel oxide nanoparticles (NPs) are one of the important metal oxide NPs increasingly used in various industrial technological and biomedicine applications. Nickel oxide NPs are used in ceramic and electronic engineering, for storage battery, condensers, as a catalyst and varistors (1, 2). In general, nanotoxicity research focused on respiratory tract exposures for assessing the health effects of metal nanoparticles. According to the researchers, nickel oxide NPs agglomerate in the lung tissues inducing pulmonary inflammation (2, 3). Although nano-sized particles can cross membranes and further distribute to other organs, there is no adequate studies about evaluating toxicity of nickel oxide NPs in renal, brain, liver, intestine system. Therefore, we investigated the cyto- and genotoxic effects of nickel oxide NPs (TEM >50 nm) in neuroblastoma (SH-SY5Y), colorectal (CaCo-2), liver (HepG2) and kidney (NRK-52E) cells. For the genotoxic effects, alkaline comet assay was used at 15-120 µg/ml. For the cytotoxic activities, trypan blue, neutral red uptake and mitochondrial succinate dehydrogenase assays were used at 62.5-500 µg/ml. In the present study, nickel oxide NPs caused cyto- and genotoxic activities with different range against the different cells. Nickel oxide NPS decreased the cell viability (IC_{50} ≥ 74µg/ml), and increased significantly DNA damage. At the highest exposure concentration, tail intensity increased approximately 6-fold compared to the control group. In future, it will be better to evaluate their cellular morphological changes and toxicity mechanism pathways such as oxidative stress and apoptosis incidence. The present study highlights on the *in vitro* toxicity of nickel oxide NPs over different targets.

(1) *Horie M, Nishio K, et al. Ultrafine NiO particles induce cytotoxicity in vitro by cellular uptake and subsequent Ni (II) release. 2009. Chem Res Toxicol.8:1415-26.*

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P4-2

NITRIC OXIDE-RELEASING POLYMERIC NANOPARTICLES AGAINST TRYPANOSOMA CRUZI

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Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, *Trypanosoma cruzi*, and the disease remains a major health problem in many Latin American countries. Several papers report that the killing of the parasite is dependent on the production of nitric oxide (NO). The endogenous free radical NO is an important cellular signaling molecule that plays a key role in the defense against pathogens, including *T. cruzi*. As *T. cruzi* is able to compromise host macrophages decreasing endogenous NO production, the administration of exogenous NO donors represents an interesting strategy to combat Chagas disease. Thus, the aims of this study were to prepare and evaluate the antimicrobial activity of NO-releasing polymeric nanoparticles against *T. cruzi.* Biocompatible polymeric nanoparticles composed of alginate/chitosan or chitosan/sodium tripolyphosphate (TPP) were prepared and used to encapsulate mercaptosuccinic acid (MSA), which is a thiol-containing molecule. Nitrosation of thiols (SH) groups of MSA were performed by the addition of equimolar amount of sodium nitrite, leading to the formation of S-nitroso-MSA-containing nanoparticles. These polymeric nanoparticles act as spontaneous NO donors. The results show the formation of nanoparticles with average hydrodynamic diameter ranging from 270 to 500 nm, average of polydispersity index of 0.35, and encapsulation efficiency in the range of 99%. The NO release kinetics from the S-nitroso-MSA nanoparticles showed sustained and controlled NO release over several hours. The microbicidal activity of S-nitroso-MSA nanoparticle was evaluated by incubating NO-releasing nanoparticles (200 - 600 µg/mL) with replicative and non-infective epimastigote, and non-replicative and infective trypomastigote forms of *T. cruzi*. In addition, a significant decrease in the percentage of macrophage-infected (with amastigotes) and NO-releasing nanoparticle-treated cells was observed. Figure 1 shows the concentration dependent *in vitro* killing of S-nitroso-MSA alginate/chitosan nanoparticles against trypomastigote forms. Taken together, our results reveal a potent toxic effect of NO-releasing polymeric nanoparticles against different life cycle forms of *T. cruzi,* indicating that the encapsulation of the NO donor S-nitroso-MSA represents an interesting approach to combat and to prevent Chagas disease.

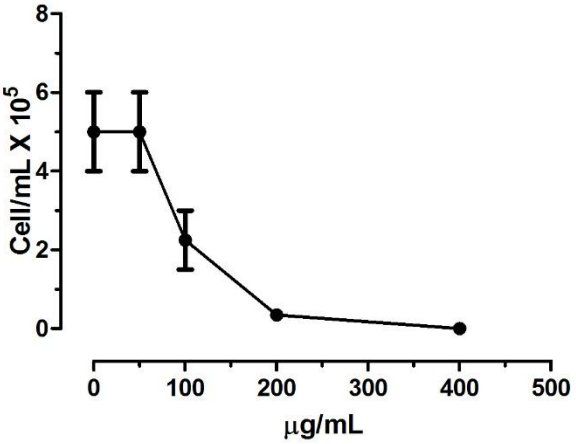

Figure 1. Concentration dependent killing of S-nitroso-MSA alginate/chitosan nanoparticles against trypomastigote forms of Trypanosoma cruzi. Number of viable cells versus concentration of nanoparticles (50 – 400 µg/mL). Incubation time: 24 h.

P4-3

CHRONIC EXPOSURE OF MOUSE TO SILICA OR TITANIUM NANOPARTICULES THROUGH DRINKING WATER RESULTS IN RENAL AMYLOIDOSIS.

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It is obvious now that the exposure to engineered nanomaterials (ENMs) is increasing with the growing variety of commercial products already available on the market of which food industry. The unique properties of ENMs have also raised concerns about their potential non intended consequences on human health and the environment. Importantly the effects on health of a prolonged exposure remain controversial.
To consider whether any long-term exposure may trigger deleterious effects, a realistic dietary exposure was designed in mice (2 lines of wild-type mice, C57BL6 expressing or not α-synuclein, a neuronal protein taking part in amyloid deposits of human neurodegenerative diseases). Mice were exposed for 18 months with drink water containing 30 µg/mL of nanoparticules of titanium (TiO_2.NP) or silica (SiO_2-NP). NPs are dispersed following the protocol developed by Nanogenotox Joint Action, in a solution made of 0.05% w/v BSA-H_2O, pH 7, undergoing 3 sonications of 24 min, at 20% of amplitude to 500W. Control experiment relies on mice exposed to tap water without NPs. At the end of the exposure, brain, liver and kidneys were removed for histological analysis. Formol-fixed, paraffin- and/or resin-embedded samples were examined after Periodic Acid Schiff stain, Masson's trichrome stain and silver-based staining. One part of each sample was also frozen in order to realize fluorescent complementary immunostaining for amyloidosis.
Here, we report the histological abnormalities observed in the kidneys which display amorphous amyloid deposits on glomeruli confirmed by specific Red Congo, Crystal Violet and Thioflavin staining. These amyloid deposits were distinctive in the mice line expressing α-synuclein, exposed to TiO_2-NP, (5/6) as well as to SiO_2-NP (1/3), while they were absent in kidneys of the exposed mice line which do not express α-synuclein, (only hyalinosis features for 3/5 with TiO_2-NP and 1/5 with SiO_2-NP). No morphological abnormalities were noted on aged-matched control mice (not exposed to NP (0/7)). Amyloidosis was associated to vacuolated tubules and interstitial lymphoid infiltrates in kidney parenchyma. The type of amyloidosis is currently under immunohistochemical characterization. The analysis in progress by ICPMS reveals already higher amount of titanium in the kidneys most severely affected. In liver parenchyma, perivascular amyloid accumulation was only observed in mice expressing α-synuclein exposed to TiO_2-NP (5/7) and SiO_2-NP (3/7).
In conclusion, the chronic dietary exposure of mice to silica or predominantly titanium NPs through drinking water triggers renal and liver amyloidosis raising a possible risk in human health after long exposure.

P4-4

IN VITRO TOXICITY OF NANOCERIA: EFFECT OF COATING AND STABILITY IN BIOFLUIDS

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Due to the increasing use of nanometric cerium oxide in applications, concerns about the toxicity of these particles have been raised and have resulted in a large number of investigations. We report here on the interactions between 7 nm anionically charged cerium oxide particles and living mammalian cells. By a modification of the particle coating including low-molecular weight ligands and polymers, two generic behaviors are compared: particles coated with citrate ions that precipitate in biofluids and particles coated with poly(acrylic acid) that are stable and remain nanometric [1,2]. We find that nanoceria covered with both coating agents are taken up by mouse fibroblasts and localized into membrane-bound compartments. However, flow cytometry and electron microscopy reveal that as a result of their precipitation, citrate-coated particles interact more strongly with cells (figure 1). At cerium concentration above 1 mM, only citrate-coated nanoceria (and not particles coated with poly(acrylic acid)) display toxicity and moderate genotoxicity. The results demonstrate that the control of the surface chemistry of the particles and its ability to prevent aggregation can affect the toxicity of nanomaterials.

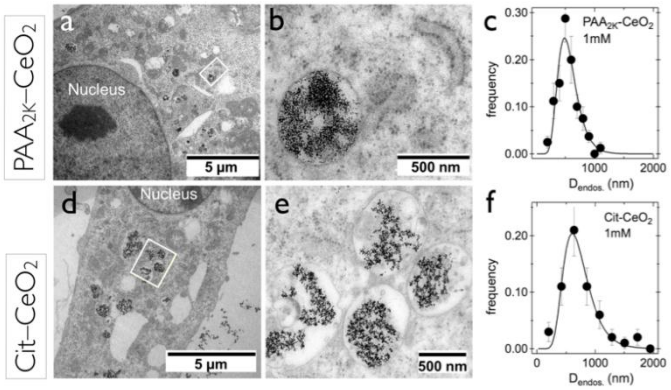

Figure 1*: Transmission electron microscopy images of NIH/3T3 fibroblast cells incubated with PAA_{2K}–Ce_2O (a,b) and with Cit–Ce_2O (d,e). The close views of the delimited areas in a) and d) show that the particles are localized in membrane-bound compartments of endosome type (b,e). The size distributions of the endosomes are shown in c) and f), respectively.*

[1] ***Safi, M.; Sarrouj, H.; Sandre, O.; Mignet, N.; Berret, J.-F.*** *Interactions between sub-10-nm iron and cerium oxide nanoparticles and 3T3 fibroblasts: the role of the coating and aggregation state. Nanotechnology* ***2010,*** *21 (14), 145103.*
[2] ***Ould-Moussa, N.; Safi, M.; Guedeau-Boudeville, M. A.; Montero, D.; Conjeaud, H.; Berret, J. F.*** *In vitro toxicity of nanoceria: effect of coating and stability in biofluids. Nanotoxicology* ***2014,*** *8 (7), 799-811.*

P4-5

MECHANISMS OF TIO_2 NANOPARTICLES GENOTOXICITY: IMPACT ON DNA REPAIR IN, A549 AND BEAS-2B EPITHELIAL PULMONARY CELLS.

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TiO_2 is a whitening agent that is extensively used in a wide range of everyday life products (cosmetics, food, paints, pharmaceuticals, etc.). This study focuses on the toxicological impact of these NPs through inhalation, using the A549 and BEAS-2B epithelial pulmonary cell lines and the commercial TiO_2-P25 NPs from Evonik®. Previous studies showed that these NPs are internalized by A549 cells and accumulate in cytoplasmic vesicles (Simon-Deckers et al., Toxicology, 2008, 253: 137-146). An acute exposure of 1-100 µg/mL during 4-48h leads to moderate cell mortality but high oxidative stress, single-strand breaks and oxidative lesions to DNA. Additionally cell ability to repair DNA was shown to be impaired after 48h of exposure, through inactivation of both NER and BER pathways (Jugan et al., Nanotoxicology, 2012, 6: 501-513). The aim of the present study was to shed light on the mechanisms underlying this impairment of DNA repair activity. The first working hypothesis was that TiO_2 NPs could impair transcription of genes encoding DNA repair enzymes and/or translation of these proteins. This hypothesis was investigated using RT-qPCR and western-blotting. Meanwhile, the possible interference of TiO_2 NPs with post translational signaling pathways is under investigation using shotgun phosphoproteomics. Our results show a global perturbation of DNA repair protein production in TiO_2-NP-exposed cells, possibly via an upstream regulator that needs to be identified.

P4-6

DEEPER PENETRATION OF TIO_2 NANOPARTICLES IN NEOPLASTIC VS. NORMAL HUMAN ORAL MUCOSA MODELS

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Introduction: Titanium dioxide (TiO_2) nanoparticles (NPs) are used in a wide range of products and could be utilized for theranostic purposes, such as the detection and treatment of cancer. Assessing the oral mucosa's barrier function with respect to NP penetration is important for both safety and theranostic reasons.

Aim: To measure and compare the depth of penetration of TiO_2 NPs in normal vs. neoplastic *in vitro* reconstructed organotypic three-dimensional (3D) human oral mucosa models.

Methods: Normal oral mucosa cells isolated from consenting healthy patients and neoplastic cells derived from an oral squamous cell carcinoma (CaLH3 cell line) were used for *in vitro* reconstruction of normal and neoplastic organotypic 3D human oral mucosa models. Both types of models used an underlying biomatrix containing type I collagen and normal human fibroblasts with either normal or neoplastic keratinocytes seeded on top. After 10 days of co-culture, the models were exposed to 5 mg/L spherical, rutile 40 nm TiO_2 NPs (American Elements®, USA) for 24 hours. TiO_2 NPs were sonicated in deionized H_2O and added to 3D model growth medium aliquots for exposure. Unexposed 3D models served as controls. After exposure, the 3D models were washed in phosphate buffered saline (PBS), fixed in 4 % buffered formaldehyde for at least 24 h then washed in PBS followed by dehydration, embedding in paraffin and then cutting into 3 µm thick sections. The sections were stained with hematoxylin and eosin and analyzed by an ultra-high resolution dark-field imaging (URI) system (CytoViva™, USA) to assess TiO_2 NPs localization and depth of penetration into the 3D models. Penetration depth was measured by using the software Olympus DP-Soft 5.0 (Olympus Corporation, Japan).

Results: URI microscopy revealed the presence of nano-TiO_2 in both normal and neoplastic human oral mucosa 3D models. The TiO_2 NPs were mainly located inside the epithelium in both normal and neoplastic models. A deeper penetration (9.56 ± 6.52 µm) was observed in the epithelium of the neoplastic models than for the normal models (3.87 ± 1.61 µm).

Conclusion: Similar localization of TiO_2 NPs was seen in both normal and neoplastic reconstructed human oral mucosa, but the penetration of the neoplastic models was deeper.

P4-7

METAL HOMEOSTASIS PERTURBATION INDUCED BY ZnO NANOPARTICLES IN HEPATOCYTE CELLS

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(1) Cuillel M, Chevallet M, Charbonnier P, Fauquant C, Pignot-Paintrand I, Arnaud J, Cassio D, Michaud-Soret I, and Mintz E. Interference of CuO nanoparticles with metal homeostasis in hepatocytes under sub-toxic conditions. Nanoscale 6: 1707-1715, 2014.

ZnO nanoparticles (NP-ZnO) are produced in high tonnage and are used in commercial applications such as antibacterial coatings or as UV absorbers in sunscreens and textiles. Recent studies also indicate that NP-ZnO may be efficient in cancer therapy, prompting us to investigate if this form of exposure to NP-ZnO elicits specific toxic effects or perturbations of metal homeostasis, in particular in the liver which is the primary organ for concentrating and metabolizing toxic agents.
In this study, we chose to investigate the toxicity of two types of characterized NP-ZnO which differ by their surface coating and their mechanism of action on hepatocytes (HepG2). As recent studies suggest that NP-ZnO toxicity may be related to their dissolution, we compared the effects of these NP-ZnO to the effects of the soluble form of zinc acetate.
We found that the NP-ZnO nanoparticles present a high cytotoxicity similar to that of zinc acetate. In order to investigate early signs, as well as chronic forms of cell toxicity, we worked with zinc concentrations of 90µM, which induced no mortality at 24 h.
We investigated the dissolution of zinc from NP-ZnO by ICP-MS measurements and microscopy. It showed a near complete dissolution of NP-ZnO in cell medium after 24h.
The expression of the genes encoding metallothioneins, heme oxygenase and ZnT1 were analysed by qPCR. All these genes are known to be involved in zinc detoxification and/or oxidative stress responses. We clearly observed a high increase in these gene expressions in both NP-ZnO and zinc acetate-treated cells.
Overall, our first results suggest that in contrast to NP-CuO for which the nanoparticulate form elicits stronger cytotoxicity (1), NP-ZnO toxicity seems a direct consequence of zinc dissolution and subsequent increase in intracellular zinc concentrations.

P4-8

ROLE OF AUTOPHAGY IN RESPONSE TO TITANIUM DIOXIDE NANOPARTICLES

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The development and the increasing production of manufactured nanoparticles (NP) are raising safety concerns because of the potential effects of NP on human health, particularly at the respiratory level. Several studies have shown that exposure to manufactured NP can induce pathogenic biological effects, with lung remodelling (fibrosis, emphysema…), depending on the physico-chemical characteristics of the NP. Currently, oxidative stress and inflammation are the most widely accepted paradigms of NP toxicity; however, the exact underlying mechanisms in the biological effects of NP still remain unknown. Autophagy is a physiological process that allows the autodigestion of the subcellar components and which is also involved in the elimination of intracellular pathogens. It has been shown that this process can negatively regulate inflammation and oxidative stress. Thus, the hypothesis of this study is that a defective autophagy could be a new mechanism explaining, at least in part, NP effects.
We choose to focus on TiO_2 NP since it is one of the most abundantly produced and widely used NP.
We used seven TiO_2 NPs presenting different physico-chemicals properties (size, crystal phase, surface embedding) and compared their effects to those of micron-size TiO_2 and carbon black (CB - for chemical composition effects). NPs were characterized by electron microscopy, dynamic light scattering and X-ray diffraction. Murine macrophages (RAW cell line) were exposed to 50 µg/ml TiO_2 and CB NP for 6 hours. Effects of NP on the autophagy process were analysed by looking at the expression of autophagy markers (LC3-II and p62) and lysosomal proteins (LAMPs and cathepsins). We also analysed the cytoskeleton network by fluorescence microscopy. Moreover, expression of inflammatory cytokines was determined in macrophages exposed to NP.
All particles, except the micrometric one, induced an increase of LC3-II protein expression, indicating an accumulation of autophagosomes. These particles also increased p62 protein level, suggesting an autophagy blockade. This perturbation of the autophagy process by NP doesn't seem to result from a disruption of the cytoskeleton but from a defect in the lysosome function as suggested by a decrease of mature cathepsins expression. Moreover, preliminary results showed that exposure to TiO_2 NP induce inflammation, at different levels depending on their physico-chemicals characteristics.
These results suggest that some TiO_2 NP, depending on their physico-chemical properties, can block the autophagy process. The future work will be to understand the mechanisms explaining this autophagy dysfunction and to determine its consequence on the toxicity induced by these NP.

P4-9

SILVER NANOPARTICLES CYTOTOXICITY – VIABILITY AND APOPTOSIS EFFECTS TO A KERATINOCYTE CELL LINE

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Silver nanoparticles (AgNPs) are one of the most commonly used engineered NPs with enhanced physicochemical and biological properties and activities, e.g. efficient antimicrobial activity. There is, however, a growing concern about their possible impact on the environment and human health. Several studies refer the toxic effects of AgNPs in vitro; however, most of the studies evaluated the toxic effects of AgNPs at relatively high doses. Therefore, it is required to evaluate the toxicity of AgNPs at low doses, i.e. below the IC50. Thus, our study aims to investigate the cytotoxicity, assessing viability and analyzing the apoptotic effects of low doses of AgNPs in the HaCaT human keratinocyte cell line as in vitro model. Cells were exposed to 30 nm citrate-coated AgNPs for 24 and 48 h, at 10 and 40 µg/mL. MTT assay results showed that, relatively to controls, the viability of exposed cells was significantly reduced upon 24 and 48 h exposure at concentrations higher than 10 µg/mL. Concerning apoptosis, for both exposure times, 40 µg/mL significantly decreased the percentage of viable cells and increased the percentage of late apoptotic cells, whereas for 10 µg/mL our results showed that only the 24 h period induced significant apoptotic effects. When comparing both exposure times, results showed that the 24 h period induced a significant reduction of viable cells for both concentrations. Nevertheless, for the 48 h period the % of early apoptotic cells was significantly higher for 40 µg/mL. Expression levels of apoptotis-related genes did not show significant differences between controls and exposed cells; however, between the two exposure times, BAX showed a significantly lower expression after 24 h for the 40 µg/mL concentration, compared to 48 h. From our data, we conclude that even low levels of AgNPs can decrease HaCaT cells' viability, and depending on concentration and exposure time also induce early and late apoptosis.

Acknowledgments
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P4-10

THE EFFECT OF DIFFERENT SIZES AND DOSES OF NANO PARTICLE ZINC ON SOME OXIDATIVE STRESS PARAMETERS IN RATS

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For study of different zinc nanoparticle dose and sizes effects on the antioxidant immunity ,10,20 and 30 nanometer particle in three doses(3,10,100) are used (orally for 28 days in 11 groups, each of them involves 5 rats).One control group and ten experimental group are treated by normal zinc and nanoparticle. Zinc nanoparticle are mixed in cellulose carboxyl methyl solution by ultrasonic for 10 minutes. For inhibition of nanoparticle action gerggan is added to suspension and it is shaked before using (wang etal.2006). At the end of therapeutic period, rates are anesthetized by chloroform and their heads are cut then cupping is done. The serums are separated by centrifuge and maintained in -80C freezer until experiment some oxidative stress indicators in serum like FRAP,TBARS and some non-enzymatic oxidative stress indicators like glutathione peroxidase and superoxide dismutase are measured.

P4-11

E171 FOOD ADDITIVE AND TITANIUM DIOXIDE NANOPARTICULE TOXICITY ON INTESTINE CELL MODELS

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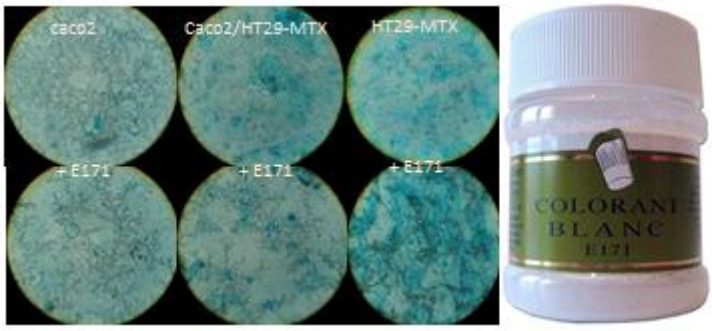
Nanoparticles (NPs) are introduced in a growing number of commercial products, particularly as food additives, in packaging or as dietary supplements. Among them, titanium dioxide (TiO_2) is widely used for controlling texture, color or moisture. Bulk and nano-scale TiO_2 is the white food color additive E171 (European Union designation). With at least 20-25% of nano-scale TiO_2 in E171 (1), it is important to study its properties and toxicity.
We previously reported that anatase, 12 nm TiO_2-NPs (A12) did not cause cytotoxicity or apoptosis but accumulated in in vitro models of M-cells (Caco-2/RajiB coculture) and mucus-secreting cells (Caco-2/HT29-MTX coculture), much less in enterocytes (Caco-2 monoculture). They induced tight junction remodelling in the regular ileum epithelium, *in vitro*, *ex vivo* and *in vivo*, which is a sign of integrity alteration and suggests paracellular passage of NPs (2).
The present study focused on the impact of TiO_2 particles on the intestinal barrier function, i.e. the ability to absorb nutrients and to exclude toxicants from the intestinal lumen. Caco-2 and Caco-2/HT29-MTX cell models were exposed to A12, but also to rutile, 20 nm NPs (R20), anatase/rutile, 21 nm (P25) and food-grade E171. These NPs were fully characterized in exposure medium. Their impact on the expression of nutrient transporters and efflux pumps was characterized by RT-qPCR and western blot. Impact of chronic exposure to these NPs on mucus secretion was then investigated. In addition, we analyzed their cyto- (WST-1), geno-toxicity (comet assay), and oxidative stress response (H_2-DCF-DA). Our results show that TiO_2-NPs alter the expression of efflux pumps and nutrient transporters, preliminary data show no impact on mucus secretion. These NPs cause no cell mortality and DNA damage, but we observe oxidative response. Taken together, these results suggest that high doses of TiO_2-NPs may alter the intestinal barrier function.



In vitro Cell models and mucus secretion after E171 (20 µg/mL) exposition during 21 days.

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(2) Emilie Brun *et al*.Titanium dioxide nanoparticle impact and translocation through ex vivo, in vivo and in vitro gut epithelia. Part Fibre Toxicol, 2014.

P4-12

IS P25 A REALISTIC MODEL TO STUDY THE TOXICITY OF TIO2 IN THE GASTRO-INTESTINAL TRACT?

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Due to its brightness and whiteness, TiO_2 is used as pigments in various applications such as paints, cosmetic and food industries. In food, it is used for a long time and especially in confectionary, where it mainly constitutes the coating of sweets and chewing-gums. It is also present in some cheeses and sauces, low-fat products such as skimmed milk and ice-creams. Unfortunately it was recently discovered that food grade TiO_2 exhibits a nano-sized fraction which can represent up to 36% of the particles. Due to the classification of TiO_2 particles as potentially harmful for humans by inhalation, the toxicity of ingested nanoparticles of TiO_2 are currently under investigation.
The toxicity of nano-sized particles of TiO_2 was until now essentially investigated with the reference particles P25, which are a kind of TiO_2 particles usually used for catalytic applications. To avoid any contest when TiO_2 particles will be reevaluated for authorization as food grade additives, it should be checked that P25 and food grade particles are identical or at least similar. In this purpose, our work compares the physicochemical properties of P25 and food grade forms of TiO_2 to decide whether P25 is a realistic model to study the toxicity of nano-sized food forms in the gastro-intestinal tract. Crystalline structures, primary particle sizes, agglomeration behaviors as a function of pH and surface reactivity, are the main properties that are compared and discussed. Some recommendations are finally addressed for further toxicological studies.
This work has been varied out in the framework of the Labex Serenade ANR-11-LABX-0064) and of the A*MIDEX project (ANR-11-IDEX-0001-02), funded by the «Investissements d'Avenir» French Government program managed by the French National Research Agency (ANR).

P4-13

GRAPHENE OXIDE SHEETS-BASED PLATFORM FOR INDUCED PLURIPOTENT STEM CELLS CULTURE: TOXICITY, ADHERENCE, GROWTH AND APPLICATION

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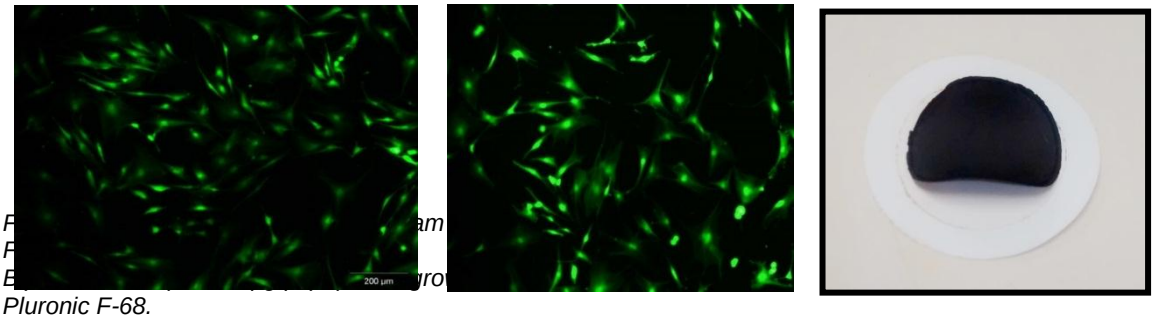
There are a few *in vivo* studies with regard to the biocompatibility of graphene oxide (GO). Previously, was developed a synthesis of thermosensitive hydrogel system based on GO by adding an amount of Pluronic (F-126 and F-68) as a physical crosslinker, without any chemical modification of GO (Sahu et al. Chem. Commun. 48, 5820 (2012)). It was reported that the gel formation was long lasting and stable and did not show any severe chronic inflammatory response. Besides this, it was found that adipose-derived stem cells (ASCs), showed increased adhesion when grown on GO films (Kim et al. J Biomed. Mater Res A 101, 3520 (2013)). However, there is still some controversy in terms of cell adhesion morphology when Mesenchymal stem cells (MSCs) are in contact with GO. Probably the inconsistency may be accounted for by the difference in cell types, the GO impurities, the substrates, and the manufacturing methods of GO. In view of this facts, it was used a representative GO from CheapTubes-USA (Go-single layer) (Durán et al., Nanosafe-2014) and ASCs from Hemocenter (UNICAMP) (Rego et al., Adv. Life Sci. EMBO-2010, 163 (2010); Luzo et al., Rev. Braz. Hematol. Hemoter. 30, 102 (2008)). Using these facts, it was prepared the GO sheets by suspension of GO in ultrapure deionized water or in Pluronic F-68 using a ultrasonicator bath. Total characterization of GO sheets was carried out. The results on suspension of GO in water showed excellent growth and cell adhesion (Fig.1).



F *am*
F
B *gro*
Pluronic F-68.

Fig.2. shows a GO/Pluronic F-68 platform for the growth and adhesion of ASCs that exhibits excellent properties for these processes. GO in water suspension exhibited an inhibition of the cell growth over 5 µg/mL. In vivo study with GO suspended in water on Fisher 344 rats via i.p. administration showed low toxicity, since they accumulates in the intraperitoneal cavity. Similar results with GO on bald/c mice were reported previously (Yang et al., Biomaterials 34, 2787 (2013)). This material was associated to chitosan in order to transform the ACSs/GO-platform to a mucoadhesive new material for treatment several diseases (e.g. Wound healing, urethritis, etc.).

ACKNOWLEDGEMENT
Support from FAPESP, INOMAT (MCTI/CNPq), NanoBioss (MCTI), Brazilian Network on Nanotoxicology (MCTI/CNPq) is acknowledged.

P4-14

SHYNTESIS, CHARACTERIZATION AND CITOTOXICITY EVALUATION OF NITRIC OXIDE-IRON OXIDE MAGNETIC NANOPARTICLES

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The present work is focused on the synthesis, characterization and cytotoxic evaluation of superparamagnetic iron oxide nanoparticles (SPIONs). We investigated the synthesis parameters, relating them with the characteristics of the produced nanoparticles in order to improve their biocompatibility, compared to the results found in the literature, and thus evaluating their applicability in nanomedicine. SPIONs have been proposed for an increasing number of biomedical applications, such as drug-delivery. To this end, toxicological studies of their potential effects in biological systems must be better evaluated. The aim of this study was to examine the *in vitro* cytotoxicity of thiolated (SH) and S-nitrosated (S-NO) SPIONs in cancer cell lines. SPIONs were prepared by the co-precipitation method using ferrous and ferric chlorides in aqueous solution. The nanoparticles were coated with thiol containing molecule cysteine (Cys) (molar ratio SPIONs: ligand = 1:20), leading to the formation of an aqueous dispersion of thiolated nanoparticles (SH-SPIONs). These particles were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM) and vibrating sample magnetometer (VSM). The results showed that Cys-SPIONs have a mean diameter of 14 nm at solid state and display superparamagnetic behavior at room temperature. Thiol groups on the surface of the nanoparticles were nitrosated through the addition of sodium nitrite leading to the formation of S-NO-Cys-SPIONs (S-nitrosated-Cys-SPIONs, which act as spontaneous nitric oxide (NO) donor). The cytotoxicity of thiolated and S-nitrosated nanoparticles was evaluated in acute T cell leukemia (Jurkat cell line) and Lewis lung carcinoma (3LL) cells. The results showed that at low concentrations thiolated (Cys) and S-nitrosated (S-NOCys) SPIONs display low cytotoxicity in both cell types. However, at higher concentrations, Cys-SPIONs exhibited cytotoxic effects, whereas S-NOCys-SPIONs protected them, and also promoted cell proliferation. Figure 1 shows the cytotoxicity of S-NOCys-SPIONs in acute T cell leukemia (Jurkat cell line) after 24 hours of treatment.

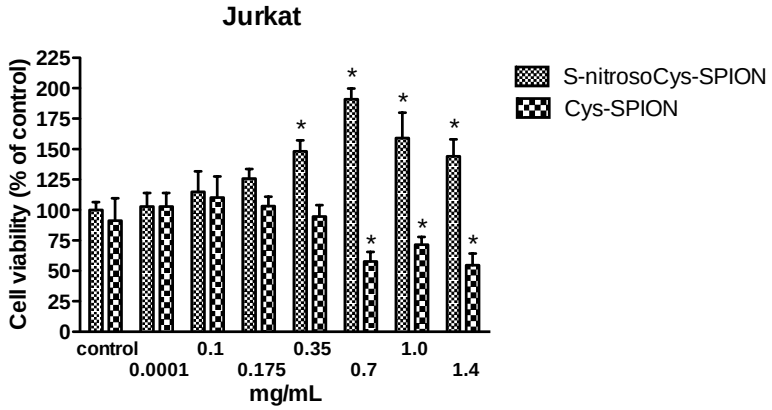

Figure 1. Cytotoxicity of S-NOCys-SPIONs in acute T cell leukemia (Jurkat cell line). Jurkat cells (1x10^5/mL) were treated with different concentrations of S-NOCys-SPIONs and Cys-SPIONs for 24 h, and afterwards, cell viability was analyzed by the resazurin reduction assay.

Acknowledgements: FAPESP, CNPq, CAPES, Brazilian Network on Nanotoxicology (CIGENANOTOX - MCTI/CNPq).

P4-15

ANALYTICAL CHARACTERIZATION OF SILVER NANOPARTICLES AND PROTEOMIC RESPONSES IN HUMAN CACO-2 CELLS AFTER ORAL INGESTION

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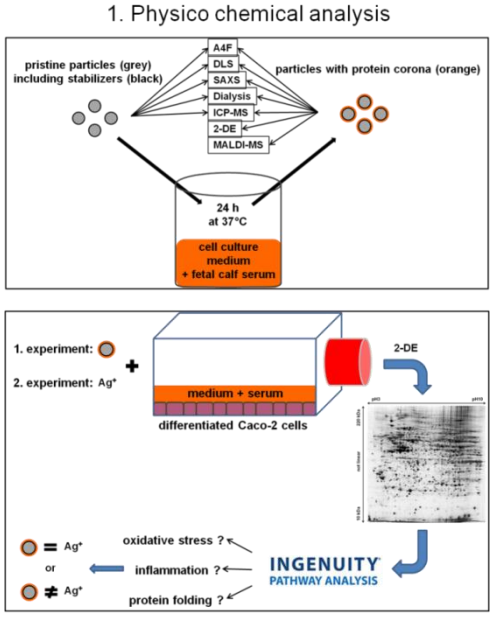
A clear molecular picture of the mechanistic effects of very good characterized silver nanoparticles is still missing. A suitable method-mix for the determination of the physico-chemical properties before and during the experiments as well as analysis of the protein corona, which will occur *in vitro*, has to be applied before interpretation of molecular effects. The aim of our study was to determine the molecular proteomics effects from a well characterized silver-nanoparticle reference material (BAM N001) and ionic silver from $AgNO_3$ on a human enterocyte cell-model (Caco-2) after 24 h incubation.
We applied asymmetric flow field-flow fractionation combined with small-angle X-ray scattering and dynamic light scattering for the determination of physico-chemical particle properties. Dialysis of the particles was employed for 24 h with silver analysis via inductively coupled plasma mass spectrometry. Subsequently, two dimensional gel electrophoresis and MALDI mass spectrometry was used for protein corona analysis as well as identification of deregulated proteins in the cells after exposure to nanoparticles and ions. Deregulated proteins were used for interpretation of signal-transduction pathways with Ingenuity Pathway Analysis.
Particles revealed negligible aggregation and an increased hydrodynamic diameter was determined after dispersion in cell culture medium caused by formation of a protein corona. The protein corona consisted mainly of albumin. The amount of released ions from nanoparticles was comparable to that of ionic silver during *in vitro* experiments. The proteomic analysis of the effect of silver nanoparticles in Caco-2 cells resulted in targets involved in inflammatory diseases, gastrointestinal cancer, ubiquitination and Nrf2 mediated oxidative stress.



P4-16

HSP70 AS AN INDICATOR OF STRESS IN THE CELLS AFTER CONTACT WITH NANOPARTICLES

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In recent years, production of nanoparticles is increased and thus grows our contact with them too. Question of safety is closely related to the issue of use nanoparticles. There are a number of tests that monitor the viability, ROS production, the effect on the DNA and cell cycle , however, rarely encountered studies on stress in the cells after contact with nanoparticles.
Heat shock proteins (HSP) are the substances with a chaperone activity. They are evolutionarily very old, conservative and they are found with a high degree of homology in prokaryotes and eukaryotes including humans. They exist at low concentrations under physiological conditions, while in the denaturing conditions e.g. high or low temperature, radiation, exposure to chemicals, heavy metals, or nanoparticles their expression is changed. HSPs are involved in maintaining homeostasis in the cell that the denatured protein conformations allow recovery to the original stage. One of the most common proteins from HSP family is Hsp70 - protein with a molecular weight of 70 kDa. The level of Hsp70 in a cell after exposure to the stress changes depending on the stress level to which the cell is exposed to and a time period during which lasted stressful conditions. Our research monitors stress levels of cells manifesting by Hsp70 production after contact with silver nanoparticles. Nanoparticles show different toxicity towards different types of target cells, which is reflected in the values of IC_{50} – concentration that kills 50 % tested cells. Concentration of test substance toxic to one cell type may be innocuous to cells of another type. IC_{50} obtained from the MTT assay provides a suitable default data and if multiples of IC_{50} values are used, we can compare and generalize. Studies can be used to compare stress levels in cells that show different sensitivity to the tested nanoparticles compared with cells under optimal growth conditions. The study was done on two types of mouse fibroblasts NIH-3T3 and L929. Exposure of cells to a temperature higher by 5 ° C than their optimum causes production of a protein Hsp70. These cells were used as positive control.

P4-17

PREDICTION OF NANO-PARTICLE PERMEATION THROUGH PULMONARY ALVEOLAR EPITHELIA BASED ON INTEGRATED USES OF A CELL-BASED *IN VITRO* MODEL AND A NUMERICAL SIMULATION

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Recent advances in nanotechnology have increased the development and production of many new nanomaterials. This is raising concerns about possible risks to human health. To establish alveolar epithelial cell-based assay systems, human epithelial cell line (A549), macrophage-like cells induced from monocytic leukemia cell lines (THP-1) by adding PMA (Phorbol 12-myristate 13-acetate) and rat primary-cultured cells (epithelial cells and macrophages) were used.
First, we investigated the cell viability after exposure to the nanoparticles (Φ21 nm TiO_2) at various initial cell densities. When the A549 cells are cultured at a low density, the drastic decrease of viability (50%) was observed. In contrast, when the A549 cells are cultured at a high density to form continuous cell monolayers, the viability decreased only slightly. Primary-cultured rat alveolus epithelial cells did not show obvious viability decreases in any cell densities. These results show that we have to carefully choose the culture conditions according to the assay's objectives such as screening with high sensitivities or elucidating mechanisms in better physiological situations.
Second, the two types of alveolus epithelial cell-based model were formed on semi-permeable membranes (culture inserts). In the case of human cell-line-based system, we were able to coculture THP-1-derived macrophage-like cells firmly adhered onto the monolayers of A549 on such culture inserts. Furthermore, as a better physiological in vitro model, we successfully formed thin monolayers (≈ 0.5 µm) of primary-cultured rat alveolus type I epithelial cells on which fresh alveolar macrophages were attached. By strictly controlling the net surface density of adhered type II cells upon isolation, the whole membrane surface was covered with type I alveolar epithelial cells after one week of culture and such thin epithelium showed very high TEER values (>500 Ω•cm^2). Primary-cultured rat macrophages phagocytized the particles more actively than THP-1-derived macrophage-like cells. This shows that by using primary cultured cell combinations, in vitro system remarkably better mimic the in vivo alveolar interface between air and liquid and that results obtained in such a primary-cultured cell-based alveolus epithelial model can be directly compare with the results obtained in rat in vivo inhalation experiments.
Finally, nanoparticles permeations were investigated using A549 and THP-1 cell-based alveolar models formed on semi-permeable membranes and relevant numerical simulation describing dynamic equilibrium among the apical side, alveolar cells, macrophages and basolateral sides. With biological kinetic parameters obtained in the cell-based assay, the numerical model largely described the concentration changes in the four compartments in the assay system. By changing some parameters such as scale of the model which is not identical to in vivo, the model would be able to describe the in vivo situation and overcome the limitations of existing culture models. Overall, it was indicated that the combination use of in vitro cell-based tissue models and numerical simulations would enable us to predict the permeation of particles through the alveolar tissue.

P4-18

INVESTIGATION OF THE POTENTIAL CYTOTOXIC EFFECTS OF ZINC OXIDE NANOPARTICLES

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Nanomaterials that are used in numerous products for personal, commercial and medical purposes, are the materials sizes from 1 to 100 nm or in some special circumstances 200 to 300 nm are also acceptable and they have specific physicochemical properties. Although they are known as today's miracle material of our life, they are attracting the attention of researchers because of their different origins, activities and toxicities. In recent studies, it has been reported that toxicitiesof nanomaterials vary depending on physicochemical parameters (particle size, agglomeration/aggregation degree, size distribution, shape, crystal structure, chemical composition, surface chemistry, surface charge, surface area, the amount of the solubility etc). In addition to the extensive useage of nanomaterials in nanotechnology field, often impurities arising during production period, differences in species and tissue can affect the toxicity (1) .

Zinc oxide (ZnO) nanoparticles has been widely used in many different industrial area, particularly in textile and food for coating and dyeing processes and in cosmetic products such as sunscreens and hair care products, due to their long term UV absorption and strong antibacterial activity (2). Some information about the studies on the mechanisms of toxic effects of ZnO nanoparticles in cells showed that in general they can cause reactive oxygen species generation, mitochondrial function damages, increases of cell and plasma membrane permeability and cell death via apoptosis or necrosis and interactions in the gene and protein level (3-4). Therefore, because of the importance of differencies in species and tissue in evalation of nanoparticles toxicity and lacking of studies on kidney epithelial cells, cytotoxic potential of ZnO nanoparticles, characterized 20-50 nm in size, were investigated in rat normal kidney epithelial cells (NRK-52E) in the present study. Mean of inhibition concentration (IC_{50}) values in cell line were 79.96 μg/ml for mitochondrial damage by MTT test. Recently in light of the literature studies, the toxic effects of nanoparticles gained importance and it is believed that the result contribute to the literature. Besides, due to frequent use of these particles and their high risk for exposure in different ways such as digestion and respiration it is really very important to evaluate the high risk of the ZnO nanoparticles that can cause cell destruction.

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4-Bondarenko O, Juganson K et al (2013) *Arch Toxicol* 87:1181–1200.

P4-19

CHARACTERIZATION OF COPAXONE® BY ATOMIC FORCE MICROSCOPY (AFM) AND DYNAMIC LIGHT SCATTERING (DLS)

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Analytical Development, Discovery & Product development, Global R&D, Teva Pharmaceutical Industries Ltd., Netanya, Israel.

Copaxone® is an immunomodulator drug used to treat multiple sclerosis. Copaxone® is an aqueous solution containing 20mg/mL of the active ingredient Glatiramer Acetate (GA) and 40mg Mannitol. GA is a complex mixture of synthetic amino acid polypeptides composed of a multitude peptide sequences containing L-alanine (Ala), L-Lysine (Lys), L-Glutamate (Glu), and L-Tyrosine (Tyr). This complex mixture of linear polypeptides results in varying chain length, therefore the molecular weight distribution of the GA components span over the range of about 2,500 – 20,000Daltons.

Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS) are two orthogonal techniques commonly used in the characterization of polymers and aggregates. AFM generates 3D topographical images of the surface ultra structure with molecular resolution and provides detailed information on the height, size, and shape of molecules. DLS measures the Brownian motion of molecules in a solution (diffusion rate) by using a laser beam. By measuring this diffusion rate it is possible to extrapolate the hydrodynamic radius and size of the molecule.
AFM and DLS methods were developed as part of efforts to characterize aggregates in Copaxone®. Using AFM Copaxone® aggregates appeared as fiber like with no globular aggregates showing good batch to batch consistency of the aggregates shape. Purported generic samples were also tested using AFM. The aggregates in these samples displayed various shapes (globular) which were dissimilar from the ones seen in Copaxone®. In addition, Copaxone® was also characterized using DLS. The DLS analysis revealed two types of populations: one population having an average hydrodynamic radius of 5nm and the other with an average of 100nm with good batch to batch consistency (as shown in the AFM analysis). The generic copies also contained two populations: one with an average dynamic radius of 5nm (similar to Copaxone®) and the other ranging from 140nm to 300nm i.e. larger than that Copaxone®. Both AFM and DLS have been proven to be sensitive and robust methods to characterize Copaxone® aggregates.

P4-20

EXPOSURE TO MANUFACTURED NANOPARTICLES DURING GESTATION: IMPACT ON THE RESPIRATORY TRACT OF THE OFFSPRING IN A MOUSE MODEL

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Due to several commercial applications of manufactured nanoparticles (NPs), such as silver (Ag NPs), titanium dioxide (TiO_2 NPs) and cerium dioxide (CeO_2 NPs), knowledge of the toxicity of those NPs is of great importance. Many studies have linked exposure to fine and ultrafine particles (from air pollution) to an increase of morbidity or mortality due to various respiratory or cardiovascular diseases. Several works have focused on the effects of pulmonary exposure to manufactured NPs. It has been shown that exposure to NPs may lead to an inflammatory response, pulmonary fibrosis and emphysema. However less is known on the effect of exposure to NPs on the offspring. It has been reported that exposure during pregnancy to TiO_2 NPs and SiO_2 NPs is associated with fetal hypertrophy, neurotoxicity, and exposure to carbon black NPs induced renal disease in the offspring. NPs may interfere with normal fetal lung development. In addition, a recent study reported that pulmonary exposure of newborn mice to TiO_2 NPs was associated with pulmonary inflammation and impaired lung development.
Therefore the aim of this study is to assess the impact of exposure by the respiratory route to various NP during pregnancy on lung development of the offspring in a mouse model, and to determine the key parameters involved in lung alterations.
For this study we used three metal NPs: TiO_2, Ag, et CeO_2 with the same size and shape, to assess the impact of NPs physico-chemical properties in the potential effects on lung development. C57Bl6/J pregnant mice were exposed weekly to 100 µg NPs by nonsurgical intratracheal instillation. Analysis (biological, histological and functional) of the lungs of the offspring were made at different time of lung development: one day before delivery (18GD for gestational day), 14 days after birth (pulmonary alveolization) and 21 days after birth (lung maturity).
Preliminary results show that the exposure to TiO2 NPs during pregnancy did not affect the number of fetuses per litter, or the weight of uterus and placenta. However the weight of fetuses whose mothers were exposed to TiO_2 NPs is significantly decreased. A decrease of VEGF-A gene expression, involved in lung development, was also observed at 18GD.

These preliminary results suggest that exposure to TiO_2 NPs during pregnancy could affect the development of the offspring. For the remainder of this work, pregnant mice will be exposed to other NPs (Ag and CeO_2) in order to see if this effect depends on the type of NP. Moreover additional analyzes will be performed to confirm whether lung alterations are observed at later times after birth, and to understand the key parameters and mechanism associated with these changes.

P4-21

CHITOSAN NANOPARTICLES; ASSESSMENT OF INTERNALIZATION AND CYTOTOXICITY IN VITRO

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Over recent years the biocompatible nanomaterials have been used in different fields and their applications generating highlight more specific and effective dosage forms able to enter and cause stimuli within the cell or organism. The market currently offers a variety of nanostructured applications in the industry, medicine and biology products, however research and methods used in their study are in development and not widely known.
In this context, knowing the potential toxicity associated with nanoparticle systems obtained our laboratory; and its potential use in different areas of knowledge is our interest. We evaluated potential cytotoxic damage induced by nanoparticles made of chitosan-based.
Chitosan is a biocompatible polymer that is derived from chitin which is found in the shells of crustacean and certain insects. Nanoparticles systems were obtained by polymerization and ionic gelation method; size and shape were characterized by scanning electron microscopy and surface charge of the nanoparticles was measured using specific Z potential. After the characterization of the nanoparticles their cytotoxic potential was assessed by the MTT and LDH viability assays, its intracellular localization on hepatic and renal cells.
Are reported and discussed in this work the results and the correlation between the physicochemical properties of chitosan nanoparticles and their cytotoxic potential.

P4-22

WHAT EFFECTS HAVE FINE PARTICLES IN THE VASCULAR SYSTEM? AN INTEGRATED PROTEOMIC AND METABOLOMIC STUDY ON HUMAN ENDOTHELIAL CELL

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The impact of nanoparticles is still poorly understood. Epidemiologic studies have indicated that environmental exposure to airborne particulate matter (primarily diesel exhaust) may promote cardiovascular diseases; however, it is not clear whether these effects are due to the exposure to these environment particles or because of its co-contaminants (i.e. iron, chromium, salts and PAHs). The precise ways of action are still unknown and the suggested mechanisms often lack the convincing evidence because they have been derived from experiments in which nanoparticles have been applied in high doses. In the present study the human endothelial cell line EA.hy926 was exposed to pure carbon black and benzo[a]pyrene loaded carbon black to mimic diesel exhaust, to determine the effects of these nanoparticles on the cardiovascular system.
To get some insight into the mode of action at rather low exposure concentrations, we adopted an extended exposure time (up to 14 days) and concentrations as low as 1 ng/mL (range 1-1,000 ng/mL), which is a magnitude of order lower than concentrations applied in nanotoxicity studies using in vitro models. Contrary to the most proposed mechanism, cytosolic ROS production seemed to be unaltered after exposure while cell proliferation was significantly enhanced. By application of multiple “omic” techniques (phosphoproteomics, whole-cell proteomics, metabolomics) we could identify various regulated proteins after exposure to the particle. The major and to some extent unexpected outcome of this study was, however, that CB and CB+B[a]P had a large impact on carbohydrate and lipid metabolism. This was accompanied by an increased expression of enzymes of the glycolysis and a decreased one of enzymes involved in the citrate cycle. The bioinformatic evaluation of the regulated proteins and phosphoproteins revealed the possible involvement of the nuclear receptor PPARγ, which was verified in an ELISA-based assay. PPARγ interacts with the transcriptional coactivator PGC-1α which described to by a key mechanism in the AgNP-exposed cells.
In a next step we further analyzed the alterations of the cytoskeleton as the phosphoproteomic approach revealed alteration of cytoskeleton protein. Overall, the staining of the actin skeleton revealed modulations and functional tests showed enhanced cell migration and invasion.
As these modulations were associated in the literature with an enhanced calcium influx, the cellular calcium content was determined with fluorescence probes (Fluo-4 and Rhod-2 for cytosolic and organelle calcium). Both calcium concentration were remarkably increased after exposure (Fig.1)

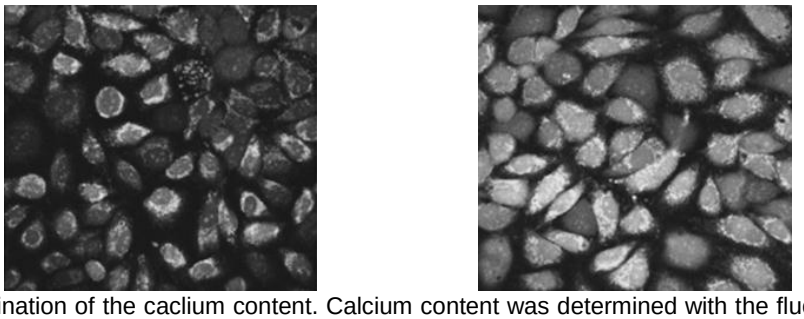

Fig. 1: Intracellular determination of the caclium content. Calcium content was determined with the fluorescence probes Fluo-4 (cytosolic Ca2+) and Rhod-2 (organelle Ca2+). Control cells are shown on the left, Carbon Black exposed cells on the right.

P4-23

CYTO AND GENOTOXICITY OF SILVER NANOPARTICLES ON MG-63 AND A549 CELL LINES

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Advances on silver nanoparticles synthesis and their unique physico-chemical properties made it useful in a wide range of sectors (e.g. from industry to medical areas). The unique size-dependent properties of nanomaterials have been responsible for technological breakthroughs, but also for increasing bioavailability and toxicity. Despite the attention around this subject there is still a lack of systematic toxicological information.
In the present work, we aimed to evaluate the cytotoxic and genotoxic effects of AgNPs and Ag^+ ion on the lung cell line A549 and bone cell line MG-63. Cells were exposed to PVP-coated AgNPs of 10 and 20 nm up to 100 µg/mL and to Ag^+ up to 20 µg/mL, for 24 and 48h. Cytotoxic effects on cell viability and proliferation were evaluated by MTT and Clonogenic assay, respectively. Genotoxic effects of AgNP were assessed by CBMN assay.
Results showed a significant decrease on cell viability after 24h exposure to AgNP and Ag^+. CBMN assay results showed that AgNPs induced clastogenic effects, chromosome damages and apoptosis for the both cell lines. A significant increase on the total amount of micronucleus was obtained for MG-63 cell line. Also, AgNP induced a high cytostatic effect on A549 cell line.
This work shows that is important to understand the AgNP mechanism of action and that each cell responds in a different way to nano toxicity.

P4-24

TOXICOLOGICAL EFFECTS OF TIO_2 NANOPARTICLES: INFLUENCE OF NANOPARTICLES CHARATERISTICS AND CELLULAR MODELS

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Titanium dioxide nanoparticles (TiO_2-NPs) are in the center of attention, because of their outstanding properties and relatively low cost of production. They have been widely used in commercial product as in pigments, sunscreens or toothpaste (1). Moreover, biomedical applications with NPs are high and TiO_2-NPs appear like a powerful applicant (2). However, the interactions between TiO_2-NPs and public health are insufficiently studied despite that OECD listed TiO_2-NPs as a priority for immediate testing. Most of the studies investigated the toxicity of commercial TiO_2-NPs on biological systems like cells in culture but few regards were accorded to TiO_2-NPs modified in form. In this context, we were able to produce TiO_2-NPs (nanoneedles, nanotubes) from commercial P 25 Evonik. These well-characterized TiO_2-NPs are different by their crystal phases, size, surface area or exposed faces. We studied the uptake and the toxicological effects of these TiO_2 nano-objects on different *in vitro* unicellular models (HUVEC, HeLa cells).
In a first step, the bio-distribution and bio-accumulation of the different forms of TiO_2-NPs after cells exposure were investigated using complementary high resolution imaging techniques. The internalization and bio-distribution of nanoparticles were studied by transmission electron microscopy. Confocal microscopy allowed us to show the co-localization of fluorescent dye-modified TiO_2-NPs and sub-cellular organelles (early and late endosome, lysosome). The bio-accumulation of TiO_2-NPs in cells was investigated by ion beam analysis (X-rays emissions). This last method permits to evaluate ionic homeostasis by imaging elemental distribution in cells and quantify chemical elements. A previous research on keratinocytes shed light on a relation between P 25 Evonik and calcium (3).
The toxicity of TiO_2-NPs was investigated by the measurement of cell proliferation during 7 days of exposure. Our results showed that the cellular response depends on the nanoparticles characteristics and the cell type. In fact, toxicity was observed in HUVEC and HeLa after the titanate nanotubes exposure according to the size and the surface area of these NPs and the concentration tested. HUVEC cells seemed to be more sensitive than HeLa whatever the TiO_2-NPs tested and the concentration. Currently, we analyzed others end-points (ROS, cell death…) to complete these first results.

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This work is supported by the ANR program “Contaminants, Ecosystèmes, Santé” (TITANIUMS) and the Region Aquitaine (TOXNANO program).

P4-25

IN VITRO EVALUATION OF IRON OXYDE NANOPARTICLES AND TITANATE NANOTUBES ON A HEPATOMA CELL LINE: CYTOTOXICITY AND GENOTOXICITY

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The development and production of nanomaterials, defined as materials with at least one dimension smaller than 100 nm, is one of the fastest growing areas of advanced technology, providing a wide range of novel applications in the electronics, healthcare, cosmetics, agronomy, engineering and food industries. The enormous development of nanotechnology has increased the nanoparticles concentration in the environment and human surroundings, causing continuous exposure, uncontrolled contact of nanomaterials by inhalation, with the skin or oral administration. Another type of exposure exists and is represented by drug injections.
Lack of toxicological data on nanomaterials makes difficult to assess the risk due to nanomaterials exposure. So, there is an urgent need to develop rapid, accurate and efficient testing strategies to assess health effect of these emerging materials.
In this perspective, a new technological platform called "NanoCare" devoted to the hazard evaluation of nanoparticles is developed within WELIENCE, a subsidiary of research development at the University of Bourgogne. This project dedicated to the development and the innovation is an opportunity to assess, in particular, the toxicity of iron oxide nanoparticles (Superparamagnetic Iron Oxide Nanoparticles, SPIONs) and titanates nanotubes (TiONts).
Superparamagnetic Iron Oxide Nanoparticles (SPIONs) have been the most extensively investigated due to their excellent biocompatibility and ease of synthesis for multifunctional biomedical applications such as cellular targeting and drug delivery, tissue repair, magnetic resonance imaging (MRI) and magnetofection. Titanate nanotubes nanomaterials have been developed for several physico-chemical applications (photocatalysis, lithium-ion batteries, gas sensor, *etc...*). Recently, they have been studied for biomedical applications too, such as dopamine detection, bone regeneration, radiosenzitization of glioblastoma cells. Moreover, titanium oxides are already used as a material in prostheses and dentures.
Cytotoxicity tests (Alamar blue, kinetics of RNA synthesis) to check cellular homeostasis disruption and genotoxicity tests (comet assay +/- formamidopyrimidine DNA glycosylase) to detect DNA damages were performed on a human cell line derived from a hepatocellular carcinoma (HepG2 line). Kinetic of RNA synthesis showed cytotoxic effect of the SPIONs. This effect was not reversible after 24h fo the concentrations of 50 and 100 $\mu g.mL^{-1}$.
The protocols for these bioassays initially used in the field of food contact packaging were adapted to nanomaterials. Two major changes were made on the iron oxide nanoparticles and titanate nanotubes to reduce their aggregation and to increase their dispersion and stability in the culture medium. To improve this, culture medium composition was modified by reducing the content of Fetal Bovin Serum (FBS). In addition, the SPIONs and TiONts suspensions underwent sonication to break up aggregates and homogenize the dispersion before their dispersion in culture medium.
Observations in transmission electron microscopy as well as confocal microscopy were performed to explore interactions at the cellular level to understand and explain the toxic effects observed. The internalization of SPIONs and TiONts was demonstrated and information was obtained about their localization in different cell structures.

P4-26

IN VIVO NANOTOXICOLOGY OF HYBRID SYSTEMS BASED ON COPOLYMER/SILICA NANOPARTICLES/ANTICANCER DRUG

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Given the potential of nanostructures for sustained release of drugs, treatments for various cancers using these materials have been widely considered and, currently, the use of nanotechnology for the development of various chemotherapies has become a valuable therapeutic option.
Among the several types of cancer, prostate cancer (PC) has a high probability of incidence, and is considered an epithelial disease that often extends beyond the limits of normal organ. In Brazil, the National Cancer Institute (INCA) estimated 60,180 new cases with 12,778 deaths from the disease in 2012. It is estimated that, currently, the risk of an north-american being diagnosed with PC cancer is 1 in 6 individuals (15%) and the risk of death from cancer is 1 in 30 (3.3%). In Brazil, the risk of death ranges close to 17/100 thousand (American Cancer Society, 2013; INCA, 2013). In view of the strategic role of chemotherapy and the need for new molecules, we highlight the association of nanomaterials and anticancer drugs, such as 14-OH-AN in the treatment of PC. 14-OH-AN is an anti-tumor broad-spectrum antibiotic with limited efficacy due to their high toxicity and side effects, which involve myelosuppression, alopecia, nausea, vomiting, cardiotoxicity. In view of these facts, this work comprises a novel formulation for prostate cancer treatment consisting of a hydrogel composed of a copolymer (POLF-120/7), anticancer drug (14-OH-AN) and silica nanoparticles (SiNP). Fig.1 shows the TEM image of the silica nanoparticles in which is possible to observe spherical and porous surface (size 50-80 nm). Fig.2 shows SEM image of the hydrogel incorporated with loaded SiNP with 14-OH-AN. The formulation is a reversible hydrogel that is liquid at low temperatures (>20ºC) and a gel in high temperatures (~37^{o}C) (e.g. human body temperature), and consequently can be used in cancer treatment via *in situ* gelation. The formulation provides a controlled release of the drug as the hydrogel is dissolved in the biological medium (intraperitoneal fluid) and the drug is released from the SiNP (Fig.3).

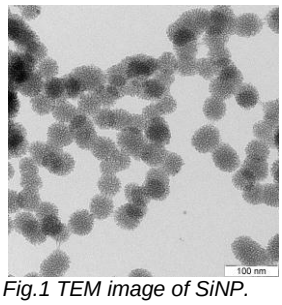

Fig.1 TEM image of SiNP.

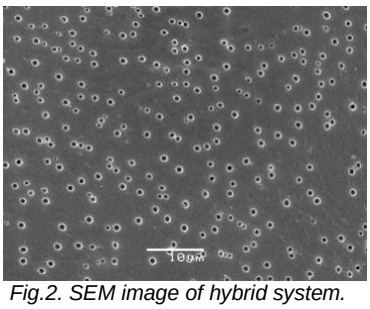

Fig.2. SEM image of hybrid system.

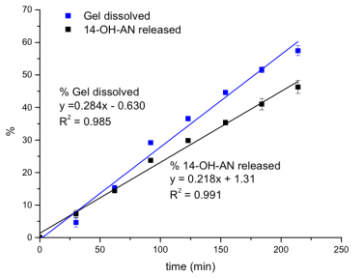

Fig.3. Disolution and release of 14-OH-AN/SiNP (ratio 0.25)

It can be seen in Fig.3 that 14-OH-AN is released as the gel is dissolved. It can also be seen that at the end of 214 min, the hydrogel-14-OH-AN reached ~60% dissolution and less than 45% of 14-OH-AN released. The differences in the dissolution curve of the gel and release of 14-OH-AN is an indication that the nanoparticles promote a sustained release of the active.
The macroscopically analyses of the animals, besides the histopathological, immunohistochemical and toxicological analyses showed that the treated animal with the system containing 14-OH-AN suppressed the tumor growth. The system without silica nanoparticles showed a macroscopic alteration of heart and side effects expected from 14-OH-AN alone. The animals treated with the system containing 14-OH-AN and SiNP did not exhibit heart macroscopic lesions, indicating a possible cardio protection of the nanoparticles. Then, it was observed efficacy in cancer treatment as well as considerable decrease of drug toxicity in rats.
Acknowledgement: Support from Brazilian Network of Nanotoxicology (MCTI/CNPq), INOMAT (MCTI/CNPq), NanoBioss (MCTI) and FAPESP are acknowledged.

P4-27

SCREENING PLATFORM FOR HUMAN HEALTH IMPACT FROM INHALATION OF AIRBORNE NANOPARTICLES

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Exposure to airborne particles in an urban and indoor environment or occupational setting can pose serious hazard to human health. An integrated technological platform, consisting of in vitro cultured lung cells, a VITROCELL® air-liquid interface exposure system, aerosol generation and online characterization instruments, and a battery of biological assays for screening of human health impact from inhalation of airborne particles have been set up at VITO.
The in vitro aerosol screening platform has successfully been applied for assessing airway effects of key oxidation products and particles from ozone-initiated limonene reactions and printer emissions in indoor office air in the FP7 Officair project. These insights were further developed for application to the in vitro inhalation testing of nanoparticles (NP). NP aerosols of 10-nm Ag and 25-nm CuO were generated and applied onto human alveolar A549 cells for two hours continuously using a newly developed VITROCELL dispersion system coupling the particle generator to the cell exposure system. Particle size distribution and number concentration were measured online for NP characterization. Cell deposited particle number concentrations were determined using nanoparticle tracking analysis. NP impact was evaluated based on established biomarkers (e.g. IL-8 production). The study methodology and first results will be presented.

P4-28

CONSTRUCTION OF A DATABASE ON NANOTOXICITY FROM PEER REVIEWED PUBLICATIONS: DATA CURATION AND IMPLEMENTATION OF ONTOLOGY.

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Although the quantity of literature on the toxicity of nanomaterials is staggering still little is known about their impact on human health and the environment. A structured integration of the multitude of available research data could lead to better understanding and assessment of the risks/benefits of nanomaterials. The Mod-Enp-Tox project aims to assess the toxicity effect of engineered nanoparticles from a joined database which combines both the physico-chemical characteristics (PCCs) of the nanoparticles and their measurable biological endpoints.
We report here the first steps in building the database collecting data from published peer reviewed papers and reports. To standardize this procedure we are working on a common SOP for data curation developed in conjunction with the USNanoGroup and the EU members of the NanoSafety Modeling Cluster. The SOP consists of information on data evaluation, data conversion into a standard format and a quality control of the procedure.
The PCCs of the nanoparticles are evaluated on their quantity and quality. Nine characteristics were defined to be key determinants of their interaction with biological systems. For each particle information on chemical composition, size and shape is obligatory to be included into the database. To increase the validity a quality score (QS) was added to each data point to specify the credibility. NanoParticle Ontology (NPO) is used to facilitate the integration, interpretation and mining of the data. Evaluation of the biological endpoints is based on the commonness of the assay and the detail of methodology description. To homogenize the data the read outs of similar assays were converted into one biological endpoint. Again here we followed – as far as possible – NPO and added a QS.
To store the data an internal format compliant with the ISA-TAB-Nano format was constructed. The format is designed to store both PCCs of the nanoparticles and their biological endpoints. Meer info..
Using this methodology we have now built a preliminary database on data extracted from 36 peer reviewed papers on nanosilica.
Besides the difficulties we encountered we learned that it is essential to create a dialogue between researchers and data analysts to develop a workable structure.

Acknowledgements: This work is part of the EU FP7 project: MOD-ENP-TOX (ID 310715) - Modeling Assays Platform "MAP" for hazard ranking of engineered nanoparticles (ENPs).

P4-29

BINDING AND UPTAKE MECHANISMS OF CHARGED GOLD NANOPARTICLES IN IMMUNE CELLS

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The determination of the influence of functionalised gold nanoparticles (AuNPs) on human cells gains much crucial information due to the various medical applications of this particle type. AuNPs of different sizes can be synthesised with specific coating molecules for stabilization and to enhance uptake. One commonly used coating molecule is chitosan, a polyglucosamine that provides a positive charge to the particle, enhancing penetration of the negatively charged plasma membrane. We have previously found that a high chitosan concentration on the AuNP surface correlates with enhanced cell death and pro-inflammatory conditions; the aim of this research was then to establish which factors have the highest impact on cellular uptake and cytotoxicity, whether it is surface charge, specific modification molecule or NP size. Therefore we assessed AuNPs of different sizes (10nm and 50nm) and different surface charges through specific surface modifications, including sodium citrate (negative surface charge), and aminoundecanethiol and chitosan (positive surface charges). It is worth noting that two of these components, AuNPs and chitosan, are often considered inert, and alone or in combination are proposed in numerous medical applications.
The uptake mechanism of AuNPs in PMA-stimulated THP-1 monocyte-like cells was investigated, with focus on transferrin-mediated endocytosis. This iron transporting protein is reported to be a capable uptake mediator for NPs, and will likely form part of the protein corona of NPs in serum. Therefore we focus on different holotransferrin conditions to determine which impact this protein has on the uptake of AuNPs and subsequent cellular responses such as cytotoxicity and inflammation. We have regulated the surface transferrin receptor (TrR) expression using hemin and desferrioxamine, evaluated by RT-PCR on an mRNA level, and by flow cytometry to assess surface presentation. In addition we conducted spiking experiments with rising concentrations of holotransferrin in serum-free medium.
To gain an insight in the inflammatory response of THP-1 cells to different transferrin conditions in combination with AuNPs, secretion levels of IL-1β, CCL2 and TNFα were assessed. In general, a greater inflammatory response and higher cytotoxicity were shown for chitosan coated gold nanoparticles when compared to sodium citrate stabilized AuNPs. However, we found that IL-1β secretion was dose-dependent on the presence of transferrin, for sodium citrate coated AuNPs and 0.01% chitosan coated AuNPs only. The same dose dependency was found for CCL2 as well as for TNFα. With the up and down-regulation of TrR we confirmed our findings of transferrin dependence, as the same AuNPs induced IL-1β secretion dependent on TrR upregulation. Our results make it likely that the TrR is involved in the uptake of at least two the AuNP types studied here, and that this uptake leads to cell death and pro-inflammatory conditions.
The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement No: 263147 (NanoValid - Development of reference methods for hazard identification, risk assessment and LCA of engineered nanomaterials).

P4-30

THE "NEW" OLD DOSE CONCEPT FOR NANOPARTICLES RISK ASSESSMENT

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In order to calculate the dose for nanoparticles (NP), (i) relevant information about the dose metrics and (ii) a proper dose concept are crucial. Since the appropriate metrics for NP toxicity are yet to be elaborated, a general dose calculation model for nanomaterials is not available. Here we propose how to develop a dose assessment model for NP in analogy to the radiation protection dose calculation, introducing the so-called "**deposited and the equivalent dose**". As a dose metric we propose the total deposited NP surface area (SA), which has been shown frequently to determine toxicological responses e.g. of lung tissue. The deposited NP dose is proportional to the total surface area of deposited NP per tissue mass, and takes into account primary and agglomerated NP.

The NP dose model includes the **deposited dose** (D) as the total deposited NP surface area per tissue mass in analogy to the absorbed radiation energy in biological tissue, the dose rate (uptake over time) and the biokinetics. **The equivalent dose** (H) takes into account the physico-chemical properties of different NPs. These physico-chemical properties are currently quantifiable and are included in the concept as NP-specific weighting factors. Furthermore, at present there are not enough data available to estimate the effective dose (E, weighted by the sensitivity of the biological material; tissue, organ, and cell), however we suggest to use specific organ/tissue/cell weighting factors when data will be available.

By using several **weighting factors** the equivalent dose additionally takes into account various physico-chemical properties of the NP which are influencing the biological responses. These weighting factors consider the specific surface area, the surface textures, the zeta-potential as a measure for surface charge, the particle morphology such as the shape and the length-to-diameter ratio (aspect ratio), the band gap energy levels of metal and metal oxide NP, and the particle dissolution rate.

The calculated equivalent doses for the different NPs show relative differences. Their actual values have to be determined in dedicated experimental investigations such as high throughput (HTP) in silico and in vitro assays. To progress systematically on the determination of weighting factors other relevant biological endpoint(s) have to be considered and tested. As it is known for ionizing radiation, data from a battery of endpoints indicate the biological effectiveness of the damaging agent. Similar approaches, by using modern HTP techniques, will allow identification of absolute numbers for weighting factors and differences on NP-equivalent doses.

P4-31

TOXICITY OF PESTICIDES AND NANOMATERIALS TO NEUTROPHILS CELLS

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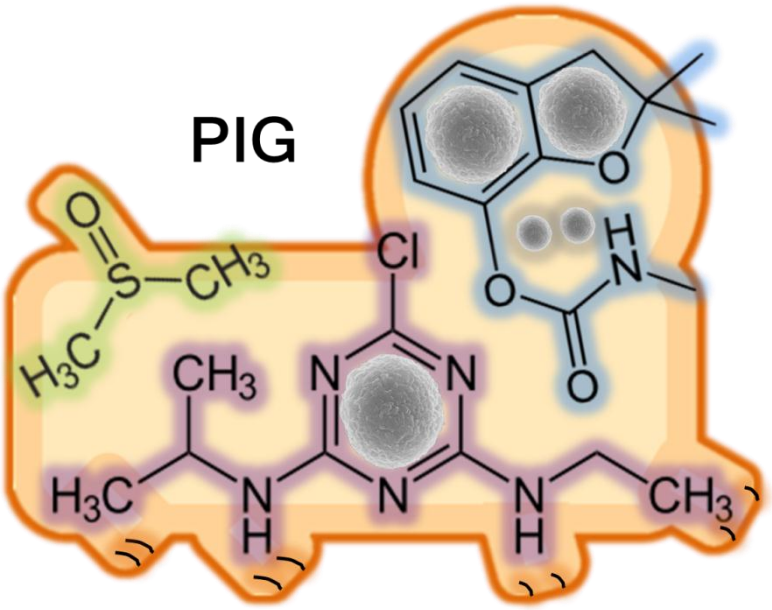
Graphical abstract



While the toxicity and ecotoxicity of herbicide, pesticide and nanoparticles have been reported over the years, the investigation of direct interactions between such chemical compounds and neutrophils have rarely been reported. In the present work, neutrophils cells were extracted from porcine whole blood and used to investigate the toxicity of different classes of toxicants that humans may contact either through the water resources or through in air. Thus, the influences of atrazine, carbofuran, Dimethyl sulfoxide (DMSO) and three types of nanoparticles (NPs) with similar sizes, aluminium oxyde NPs (< 50 nm), copper NPs (< 100 nm) and nickel NPs (< 100 nm) on neutrophils have been studied using "*in vitro*" **chemiluminescence** experiments. Experimentally, several concentrations of toxicants dissolved or suspended in water or DMSO were incubated with neutrophils for different periods of time. The survival rate and morphology of neutrophils were observed using optical microscope and scanning electron microscope, respectively. Interestingly, it has been found that results are highly dependent on several parameters such as the donor of the blood, type of toxicant, evaporation of aqueous solution from the microtiter plate wells, temperature etc. Moreover, the interaction time between toxicant content and cells played a significant role in the evolution of chemiluminescent signal kinetics. It was also founded that insoluble toxicants (nanomaterials) had stronger effects on neutrophils cells when compared to the soluble chemicals (atrazine, carbofuran and DMSO). Additionally, the experimental results are expected to be compared with the modeling outcome performed by **USEtox** methodology which is considered as a scientific consensus to assess the toxicity of a product.

Session 5
ENVIRONMENTAL INTERACTIONS OF NANOMATERIALS

P5-1

INTERACTIONS AND TOXICOLOGY OF SILVER NANOPARTICLES IN AQUATIC ECOSYSTEMS

Ester Artells[1,3,4], Clément Levard[2,3,4], Julien Issartel[1,3,4], Mélanie Auffan[2,3,4], Alain Thiéry[1,3,4] (1) IMBE UMR-CNRS 7263, Aix Marseille Université, Université d'Avignon, Aix en Provence, France (2) CEREGE UMR 7330 CNRS-Aix Marseille Université, Aix en Provence, France (3) iCEINT, Int Consortium Environm Implicat Nanotechnol, Aix En Provence, France (4) SERENADE Labex Serenade, Aix En Provence, France.

In the past 20 years, recent advances in nanotechnology have resulted in the generation of various nanostructured materials, which have unique physical and chemical characteristics. The engineered nanomaterials (ENMs) production is increasing rapidly and products containing NPs have been introduced in our daily lives. However, the environmental release of NPs, the accumulation of these new pollutants in hydrographic basins and their effect on aquatic organisms are currently unclear. Understanding the toxic effects of these emerging xenobiotics is therefore crucial in order to anticipate the consequences of the potential degradation of ecosystems and their potential impact on health[2,3].
Silver nanoparticles (AgNPs) are the most extensively used nanoparticles in consumer products. In 2012, the worldwide AgNPs outpout was estimated to be more than 10 000 tons/year[4]. More than 400 silver nanomaterials have been incorporated in day life products principally because of their antibacterial properties (textiles, cosmetics, air sanitizers or medical instruments) [5]. During manufacture, use or end of life of these products, a significant amount of NPs can be released into the environment; consequently, knowing the environmental transformations of AgNPs and their impacts on the organisms is important to anticipate their potential toxicity. In order to use "safer by design" NPs, this project propose a multiapproach annalysis regarding the potential modifications of the AgNPs in the environment by physico-chemical analyses (DLS, ICP, NMR, TEM…), and biological and toxicological analyses (survival studies, histology, gene expression, protein interactions…).
This work is part of the SERENADE (Safe(r) Ecodesign Research and Education applied to Nanomaterial Development) project whose goal is to create a dynamic network of academic research laboratories and industry to design tomorrow's nanomaterials that are safer for both humans and the environment.

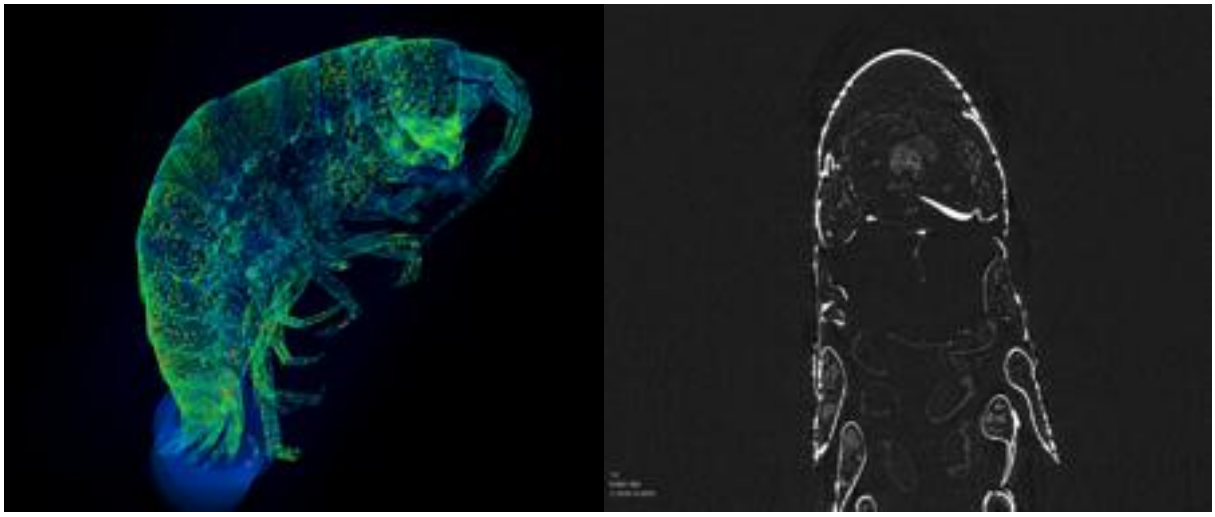


Figure 1. *Gammarus fossarum after 96 h in 500 ug/L of SiO2 NPs observed using X-ray micro-tomography*

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P5-2

SIZE-DEPENDENT TOXICITY OF BARIUM TITANATE TO *CHLORELLA VULGARIS*

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Studies have been demonstrating that smaller particles can lead to unexpected and diverse ecotoxicological effects when compared to those caused by the bulk material. Among the materials that have been studied in the hopes of finding new applications when in small size, barium titanate (BT) has been gaining momentum for biological applications as nanocarrier for proteins, uptake enhancer of low molecular weight drugs such as doxorubicin, biomarker for imaging probes and in the design of bone graft material. However, studies on its impact on the environment are lacking. In this study, the chemical composition, size and shape, state of dispersion, and surface charge, area and physicochemistry of micro (BT MP) and nano barium titanate (BT NP) were determined. Green algae *Chlorella vulgaris* grown Bold's Basal (BB) medium or Seine River water (SRW) was used as biological indicator to assess their aquatic toxicology. Responses such as growth inhibition, cell viability, superoxide dismutase activity, adenosine-5-triphosphate content and photosynthetic activity were evaluated. Tetragonal BT (~170 nm, 3.24 m2 g−1 surface area) and cubic BT (~60nm, 16.60 m2 g−1) particles were negative, poorly dispersed, readily aggregated, and precipitated in both SWR and BB medium. As for the aquatic toxicology: (i) BT has a statistically significant effect on *C. vulgaris* growth even at the lower concentration tested (1ppm), what seems to be mediated by induced oxidative stress caused by the particles; (ii) the BT behavior was different when in synthetic or in natural culture media, the toxic effects in C. vulgaris being more pronounced when grown in SRW (in this case, a worse physiological state of the algae growing in SRW can occur and account for the lower resistance, probably linked to a paucity of nutrients or even to a synergistic effect with a contaminant from the river); and (iii) size does not seem to be an issue influencing the toxicity in BT particles toxicity since micro- and nano-particles produced significant effects on algae growth – although the growth inhibition was more pronounced with the nanomaterial.

P5-3

SILVER NANOPARTICLE TOXICITY TO *PSEUDOMONAS PUTIDA* MONOSPECIES BIOFILMS UNDER FLOW CONDITIONS

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Extensive and poorly regulated use of engineered nanoparticles has raised concern regarding their potential release in (and impact on) the environment. Despite being a growing area of research, general knowledge and understanding of nanoparticle fate, bioavailability and toxicity is still limited. Bacterial bioreporters have been extensively used for drug and chemical testing over the last two decades and are now being applied in nano(eco)toxicology as well, usually via the indirect measurement of emitted luminescence/fluorescence from genetically modified microorganisms, especially *Escherichia coli,* used as planktonic cultures. However, although bacteria in the environment are more likely to occur in surface or interface associated biofilms (*i.e*. heterogeneous and resistant communities of organised and communicating cells embedded in a complex extracellular matrix), information on nanoparticle potential effect on biofilms remains scarce. In this study the toxicity of the OECD model silver nanoparticle Ag NM-300K has been assessed on *Pseudomonas putida* biofilms under flow conditions. Biofilms were grown in artificial wastewater medium supplemented with 0.5 % (w/v) of glucose as sole carbon source under laminar flow conditions, then exposed to various concentrations of Ag NM-300K NPs (0, 0.01, 0.1, 1, 10 and 100 mg/L) for 24 h. Characterisation of biofilms was performed by confocal scanning laser microscopy (CSLM, after staining with BiofilmTracer dye mix) before and after exposure to Ag NM-300K NPs. CSLM images show that biofilm morphology was altered variably depending on tested Ag NM-300K concentration (Fig. 1). Processed data including biomass, maximum and mean thickness, roughness, surface area, surface area / biomass from CSLM images will be presented and discussed.

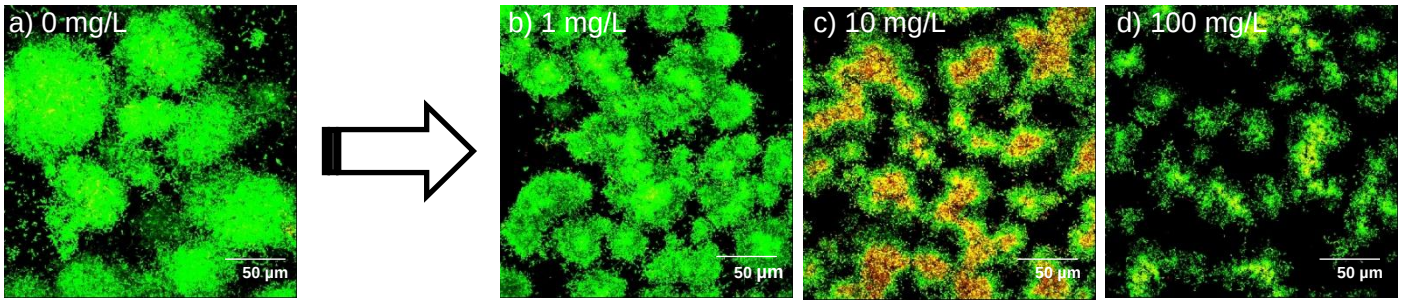

Fig. 1. Example of CSLM images after 24 h exposure to Ag NM-300K nanoparticles.

P5-4

CARBON NANOTUBES ENHANCED THE LEAD TOXICITY ON THE FRESHWATER FISH: HISTOPATHOLOGICAL EFFECTS IN THE GILLS

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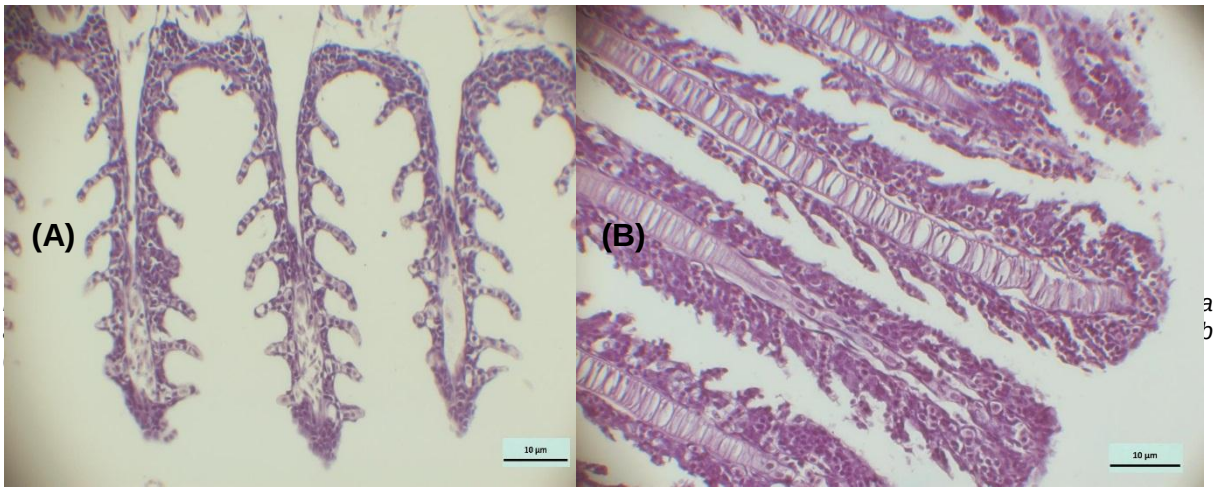
Carbon nanotubes (CNTs) are strategic materials for technological innovation. However, the safety-related aspects of CNTs to human health and environment are not well understood so far. The interactions of CNTs with classical environmental pollutants (e.g. heavy metals, PHAs, pesticides) is an important point to be addressed. Our group have recently demonstrated that oxidized multiwalled carbon nanotubes, termed HNO_3-MWCNT, enhances the lead (Pb) acute toxicity on the freshwater fish Nile tilapia (*Oreochromis niloticus*) by increasing over five times the LC50 value of Pb after nanotube interaction. HNO_3-MWCNT also decreased the oxygen consumption and ammonia excretion on the Nile tilapia after Pb interaction (Martinez et al., 2013 *J. Phys.: Conf. Ser.* **429** 012043 – Nanosafe 2012). Fish gills have a large external contact surface area and they are particularly sensitive to chemical and physical changes in the aquatic environment. In this work, we evaluated the histopathological effects in the gills of Nile tilapia juveniles after Pb, HNO_3-MWCNT and Pb plus HNO_3-MWCNT acute exposure (96h). The histopathological effects in the gills were measured according to the histological alteration index (HAI) and the average value of alterations (AVA) methodologies (Schwaiger et al., 1997 *J. Aquat. Ecosyt. Stress Recovery* **6** 75-86). Moderate histopathological effects in the gills were observed to Pb (1.0 mg/mL) and HNO_3-MWCNT (1.0 mg/L) acute exposure. However, intense and severe histopathological effects were observed in the gills of Nile tilapia after Pb-nanotube interaction (i.e. hypertrophy of the epithelial cells, dislocation of the epithelial cells, hyperplasia of the epithelial cells and partial fusion of the secondary lamellae) **(Figure 1).** These results draw attention for the implications of oxidized CNTs released in the aquatic environment.



P5-5

FATE AND BEHAVIOR OF SILVER NANOPARTICLES IN SIMPLE AND COMPLEX MATRICES

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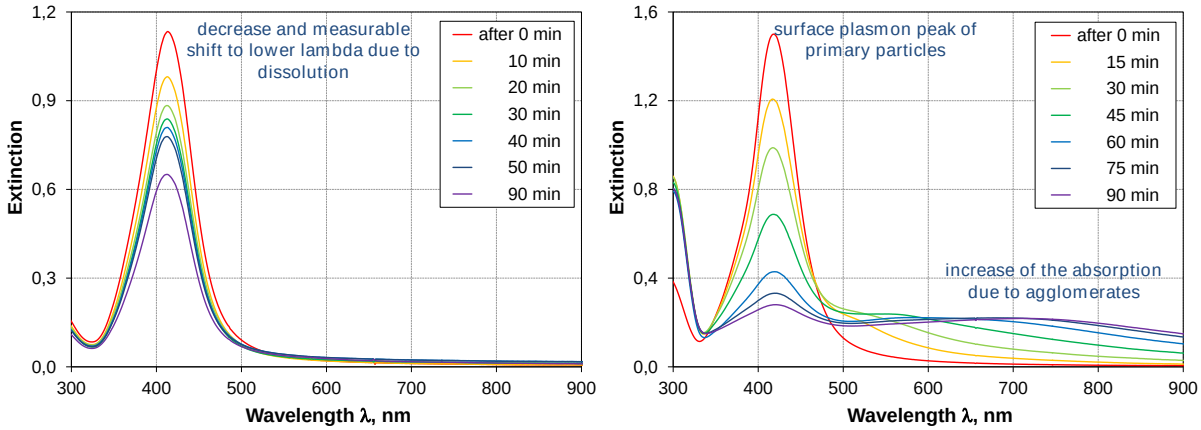
Due to the antimicrobial properties of silver ions an increasing use of engineered silver nanoparticles (Ag-ENPs) in consumer products like sportswear can be observed. This coincides with an increasing release of such nanomaterials into the environment e.g. by abrasion or washing processes. Therefore, this study examines the fate and behaviour of three differently functionalised Ag-ENPs in soil media. One of which is sterically stabilised, while the two others are electrostatically stabilised in aqueous solution. For this purpose, laboratory measurement methods for the detection of nanomaterials, like dynamic light scattering and scanning electron microscopy, and additionally uv/vis-spectroscopy were employed. The latter method is a fast and robust technique for the evaluation of Ag-ENP-suspensions, since the surface plasmon resonance of silver causes a characteristic extinction spectrum, which depends on size and shape of the particles as well as on their physical chemical properties and those of the surrounding media.
The fate and behavior of Ag-ENP was analyzed in simple ionic background and complex environmental solutions. For the environmental analysis a soil-water mixture according to the OECD guideline 106 „Adsorption-Desorption using a batch equilibrium method“ was employed. After defined shaking the mixtures were treated in several purification steps (including centrifugation and filtration) to reduce the coarse and sub-micrometer ingredients and to obtain several clear solutions with dissolved substances (e.g. electrolytes, humid acid), especially.
The uv/vis-spectra show two effects: i) a reduction of particulate silver in consequence of dissolution to silver ions, which precipitate in the presence of chlorine to hardly soluble AgCl complexes, and ii) an agglomeration of the Ag-ENPs – see Figure. The sterically stabilized Ag-ENPs appeared to be significantly more stable than the charge stabilized Ag-ENP systems. The behavior of Ag-ENPs in complex media like soil-water mixtures also depends on the composition of the soil.



Effect on extinction as consequence of dissolution (left) & agglomeration (right) of silver nanoparticles

P5-6

LICHENS AS BIOMONITORS OF CNT AEROSSOLS: A POSSIBILITY?

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Carbon nanotubes (CNT) is one of the more abundant nanomaterial produced. Therefore, it is desirable to access the effect of its presence in all environment compartments. This study aims to verify the potential use of lichens - classical indicators of atmospheric pollution - as biomonitors of carbon nanotubes aerosols. In order to examine cause-effect relationships preserving environmental microclimatic parameters, the lichen *Parmotrema tinctorum* (Nyl.) Hale was transplanted to open top chambers coupled with a suspension unit where CNT were daily added. Samples were exposed to an enriched atmosphere of 0.01, 0.1 and 0.5 grams of MWCNT per m^3 during 7 days. To observe the chronic effects, groups of samples were continuously treated during 21 days. The chlorophyll "a" fluorescence emission (pulse-amplitude-modulated fluorimeter Mini-Pam) and electrical conductivity (measured using a digital conductometer) were analyzed. No dose-dependent response was observed comparing the 3 different concentrations. The photosynthetic efficiency, expressed by the Fv/Fm ratio, and the ion leakage were not affected by the treatments. These results are in agreement with a previous study concerning the lichen responses to MWCNT-COOH exposition. The bio-distribution of CTN in lichens will be further investigated.

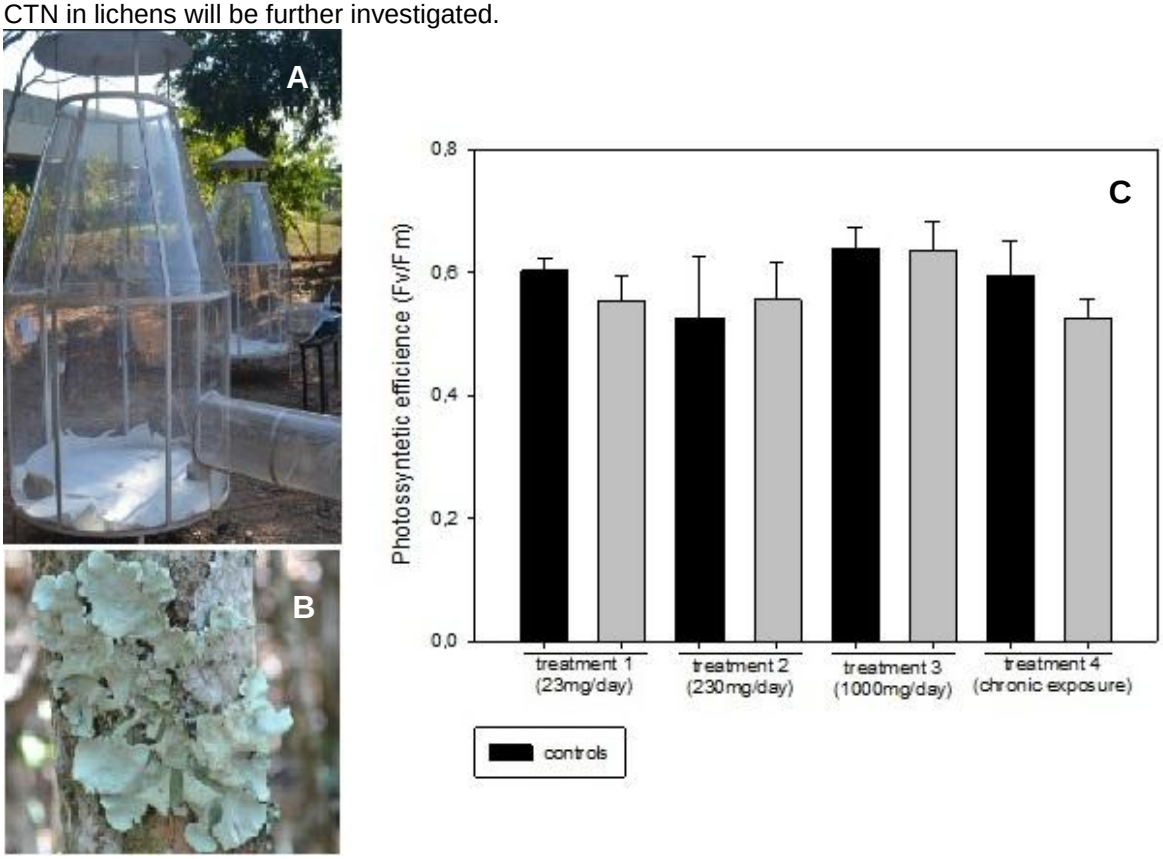

Figure 5 - A) Open top chambers; B) Parmotrema tinctorum lichen; C) Photossynthetic efficiency results

P5-7

FATE AND TRANSPORT OF ENGINEERED NANOPARTICLES ALONG THE EXPOSURE PATHWAY WASTEWATER – SLUDGE – PLANT

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The potential risks accompanying the rising use of nanotechnology are in dire need of a careful assessment. Wastewater treatment plants (WWTP) are in a key position for managing the potential risks of nanoparticles (NP) load in urban and industrial wastewater. They have to deal with the specific conditions of NP polluted wastewater and have to tackle the task of removing NP from the purified water to guarantee maximum safety of the WWTP effluent for the environment and humans. At the same time, WWTPs can potentially act as sources of NP release through the secondary uses of WWT sludge in agriculture and landscaping.

The recently started project “nanoSuppe” aims on the development of a conclusive picture of NP behaviour in WWTPs and their further fate in potential sludge uses up to the possible reintroduction in the food chain by uptake in plants. To reach this goal a strong international consortium from WWTPs, related industries, governmental agencies and research centres is formed. The project is focused on engineered NPs such as TiO_2, CeO_2, multiwalled carbon nanotubes (MWCNT) and quantum dots which might reach wastewater treatment plants e.g. through the use of consumer products (such as sunscreen) or industrial processes.

The research strategy is comprised of a thorough characterization of NPs in WWTPs from lab to field scale, including the development of predictive models of the exposure and the impact on society and environment. In this context, typical scenarios of municipal and industrial wastewater treatment technologies are evaluated and their impact on the fate of NPs with various degradation and modification levels is investigated. Furthermore, the bioavailability and the possible introduction of NPs into the food chain from the agricultural use of sewage sludge (typical used as fertilizer or for landscaping) is investigated by studying the NP extractability from soils and sediments as the crucial parameter for environmental mobility and transport of NPs and the uptake in and toxicity to various agricultural plants such as cultivated radish.

For evaluation of the transport and behaviour of NPs in highly complex media such as wastewater, sludge or plants, a reliable and sensitive detection method is the crucial parameter. Therefore, radiolabeling strategies for the NPs under study are developed. The use of radiolabeled NPs ensures identification, localisation and quantification of NPs even under the anticipated low environmentally relevant concentrations despite the highly complex media (waste water, sludge, soil, plant) and background levels of natural NPs, colloids or substances of the same elemental composition. For MWCNTs, detection in environments with a high carbon background can be realised.

Within this presentation, research strategies, project partners and first results from the collaborative project “nanoSuppe” are presented and open for discussion.

P5-8

NANOMATERIALS AS POTENTIALLY SAFER ALTERNATIVE TO FLAME RETARDANTS OF CONCERN – A COMPARATIVE HAZARD ASSESSMENT

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Flame retardants (FRs) are used as additives in a wide range of products to inhibit the spread of fire. They can act in different ways, from interfering with fire's ability to consume oxygen, to forming a barrier or acting as chemical coolants.
Some FRs, especially halogenated FRs, have been identified as global contaminants and are associated with adverse health effects including potential reproductive, developmental, and neurological effects and can be persistent and bioaccumulative [1]. Some brominated flame retardants have been banned from use and some are on the list of substances of very high concern (SVHC) for which safer alternatives need to be found.
A variety of halogen-free flame retardants are available on the market, including organic (phosphorous and nitrogen based chemicals) and inorganic (boron and metals) materials [2]. However, high loadings of these materials may be required for achieving effective flame retardancy potentially leading to modification of mechanical properties and processing difficulties (incorporation into polymers). Nanomaterials such as carbon nanotubes (CNT) and nanoclays have been demonstrated to act as very effective/synergistic co-additives in some FR applications and could thereby contribute to reducing the loading of FRs and improving their performance. CNTs have, for example, been suggested for use in textiles or polymer composites [3, 4].
In this study we evaluate and compare intrinsic properties affecting the hazard potential of selected flame retardants with focus on acute and chronic toxicity, persistence and bioaccumulation. The influence of the CNTs and co-additives on the formation of smoke particles, toxic gases (CO, HCN), and other fire effluents (such as polycyclic aromatic hydrocarbons) from burning CNT-polymer composites, under different fire scenarios, will be also assessed.
This study is performed as part of the FP7 project DEROCA (Development of safer and more Eco-friendly flame Retardant materials based on CNT cO-additives for Commodity Applications; grant agreement n° 308391), which also includes the development of new flame retardant materials and prototypes, material properties assessment, exposure assessment, life cycle analysis, and burning behaviour.

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P5-9

CHRONIC CONTAMINATION OF AQUATIC MESOCOSMS BY AG NANOPARTICLES WITH DIFFERENT SHAPE

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The enhanced physicochemical properties of manufactured (NPs) make them highly attractive for a large range of applications. Because of their antimicrobial and antifungal properties, silver nanoparticles (Ag^0-NPs) have numerous uses and are likely to enter in aquatic environments, potentially causing adverse effects not only on humans but also on aquatic ecosystems. Indeed, the oxidative dissolution of Ag^0-NPs in oxic waters produces Ag ions leading to cell death. Other possible mechanisms of toxicity are oxidative stress generated by the formation of reactive oxygen species (ROS) at the surface of the Ag-NPs and “particle specific” effects from Ag-NPs. Unfortunately, most of the toxicity studies published in the literature do not mimic the complexity of natural environments. Many national (e.g. ADEM Afsset and specific ANR) and international programs are devoted to this issue to determine rapidly if these intense uses are feasible. Because of the susceptibility of Ag^0-NPs to transform (changes in aggregation state, oxidation state, precipitation of secondary phases, sorption of (in) organic species), it is important to assess the toxicity of the Ag^0-NPs in environmentally relevant conditions. As an example, Ag^0 has a strong affinity for reduced sulfur (both organic and inorganic) and the sulfidized Ag^0-NPs showed limited acute toxicity compared to non-sulfidized Ag-NPs due to a decrease in their solubility. Chloride can also strongly affect Ag^0-NP solubility and therefore their toxicity. As a consequence, characterization of Ag^0-NP transformation and impact under realistic environment conditions is essential to understand and predict their fate and toxicity in natural aquatic systems.
In this context, to assess the environmental risk of Ag^0-NPs in environmentally relevant conditions we used intermediate size (60 L) indoor aquatic mesocosms to study the distribution and impact of two Ag^0-NPs (plates and spheres) following multiple dosings (chronic contamination). The aquatic environment mimicked was a river ecosystem with gammarus and stoneflies as primary and secondary consumers respectively. Another challenge was to work with NPs concentrations representative of what it is expected in natural aquatic environments (50 µg L-1 of Ag^0-NPs). This study highlighted that the exposure of the organisms and the impacts were not related to the shape of Ag^0-NPs. The impacts toward micro- and macro-organisms will be discussed in term of microbial diversity and oxidative stress.

P5-10

ECOTOXICOLOGY STUDY OF MAIN NANOFILLERS USED IN PACKAGING MATERIALS

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In recent years, rapid development of Nanotechnology has gained public attention as applications of nanomaterials in many areas, including industry, biomedical science, agriculture and public health, are increasing rapidly. In packaging industry, nanotechnology has gained great interest as well.
Nanomaterials have been identified as promising materials for the development of different packaging materials. Nanocomposites are polymers reinforced using organic or inorganic nanometer-sized phase (nanofiller). As fillers, NMs are able to improve mechanical, thermal, barrier and other functional properties of polymeric packaging materials.
Environmental exposure to materials at the nanometer scale is inevitable as their production and use increase, becoming part of our daily lives. It has raised concerns that their release into the environment may pose a serious threat, but research findings on their potential environmental effects are yet limited.
The main goal of this work is to provide a better understanding of the ecotoxicity of some nanomaterials, selected according to their broadest commercial interest for application in the packaging industry as nanofillers. Selection includes: metal (Ag) and metal oxides (ZnO, SiO_2).
Physicochemical characterization was carried out for nanofillers at solid state and test mediums. For ecotoxicological characterization, invertebrate organisms living in the tree main environmental compartments have been tested, including *Daphnia magna* (freshwater), *Brachionus plicatilis* (marine/esturine) and *Heterocypris incongruens* (freshwater sediment). Optimized acute/short chronic tests has been carried out, based on standardized tests, ISO 6341:2012, ASTM E1440-91 and ISO 14371, respectively. As these standards are well established for traditional chemicals, require some modifications to account for nanomaterial's particular behavior. Toxkits™ from MBT Inc. (Gent, Belgium) were employed for carrying out ecotoxicological assessment.

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Acknowledgment

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P5-11

INTERACTION OF CARBON NANOTUBE AND CELLULOSE NANOFIBER WITH ALGALCELLS KLEBSORMIDIUM FLACCIDUM

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Multi-walled carbon nanotubes (MWCNTs) and cellulose nanofibers (CNF) are noteworthy nanoparticles (NPs), which encompass a number of potential applications, being used in water treatment, cosmetics, as well as reinforcement materials, biosensors and medical equipment. However, with the rise of nanotechnologies, the risk of contamination of aquatic ecosystems with NPs is increasing. Thus, the aim of this study was to evaluate the MWCNT and cotton CNF toxicological effects on freshwater green microalgae *Klebsormidium flaccidum*. *K*. *flaccidum* was grown in sterile *Bold's Basal* (BB) culture medium at pH 7.4 at a controlled temperature of 20.0 ± 0.5°C and luminosity of 50-80 µmol m^{-2} s^{-1} photosynthetic photon flux. Appropriate concentrations of each nanomaterial stock solution (1, 50 and 100µg ml^{-1}) were added to a microalgal culture in the exponential growth phase and incubated for 24, 48, 72 and 96 hrs. Cell viability was measured by a trypan blue dye exclusion test. In order to evaluate morphological, cellular ultrastructure changes and interaction between NPs and *K*. *flaccidum*, we analyzed microalgae cells by Scanning electron microscopy (SEM) after 48 h of contact with MWCNT and cotton CNF (100 µg ml^{-1}). Data were analyzed by ANOVA and differences among means were compared by the Student–Newman–Keuls' test using the general linear model by SAS version 9.1. Differences between different groups were considered statistically significant at $P < 0.05$. NPs significantly decreased cell viability ($P < 0.05$), depending on concentration and time (Fig. 1). The cell shrinkage was noted on the cells treated with both MWCNTs and cotton CNFs (Fig. 2B and 2C). In conclusion, we have demonstrated that exposure to MWCNTs and to cotton CNFs affects cell viability and algal cell morphology.

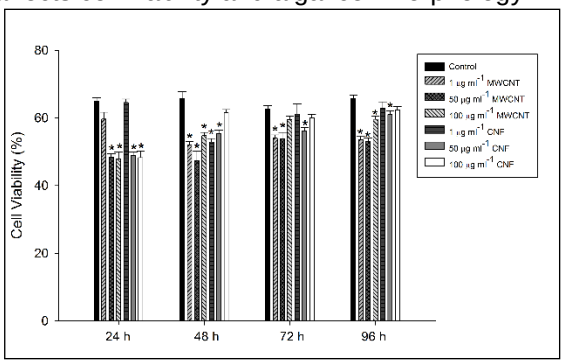

*Fig. 1 – Effect of MWCNTs or CNF on cytotoxicity of K. flaccidum in vitro cultured in BB medium. *Asterisks denote a significant difference from the control group. Calculated probability (*P < 0.05).*

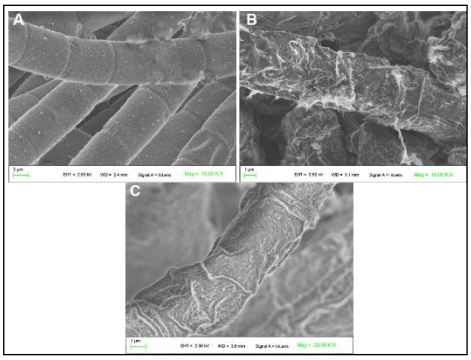

Fig. 2 – SEM images of K. flaccidum exposed to 100 µg mL^{-1} MWCNTs or cotton CNF for 48h in BB medium. A: Control; B: MWCNTs; C: CNF.

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P6-1

EVALUATION OF THE INFLUENCE OF NANO-OBJECTS IN THE REACTION TO FIRE PROPERTIES OF CONSTRUCTION PRODUCTS EXPOSED TO ACCIDENTAL FIRE

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There are few studies in the literature of the measured o the influence of the addition of nanomaterials in the reaction to fire of products[i]. In the SCAFFOLD project, an aproximation to the assessment of the risk associated with the exposure of construction products containing nano-objects to an accidental fire has been studied. Different construction materials modified with nano-objects were employed for comparison (FRPANEL: Glass reinforced composites modified with nanoclays, MORTAR: Mortar modified with nanoTiO_2, CONC: Concrete modified with nanoSiO_2, FOAM: PUR insulation modified with nanocellulose, COM: Glass reinforced Composite modified with carbon nanotubes). Cone calorimeter and smoke density chamber were used as fire scenarios. The products were tested according to the standards of each equipment and the main parameters related with fire reaction properties (Maximum average of heat release, MARHE; smoke opacity, Ds; toxic gases concentration, CITg and the presence of nanoparticles in the effluents) were provided.

In an attempt to simplify the results and to quantify the risk associated with each parameter, the following criteria were taken into account:

PARAMETER	RISK LEVEL			
	LOW	INTERMEDIATE	MODERATE	HIGH
MARHE	0-75	75-150	150-225	>225
Ds	0-150	150-300	300-600	>600
CIT_8	0-0.1	0.1-0.3	0.3-0.5	>0.5
Nano-objects	Evidences		No evidences	
⇩	Presence of nano-object improve the parameter			
⇧	Presence of nano-object worsen the parameter			

For each parameter, a color code5 was defined with the absolute values associated with the measurements: green for low risk, yellow for intermediate risk, orange for moderate risk and red for high risk. Both reference materials and nano-objects treated materials have been classified according to these criteria. In the cases that the presence of nano-objects improves the fire reaction parameter a green narrow is included. Conversely, a red narrow indicates a worsening of the parameter.
Therefore, the main data obtained in the study are summarized in the following table:

Ref.	FIRE PROPAGATION		SMOKE DENSITY		PARTICLES IN EFFLUENTS*		SMOKE TOXICITY	
	MARHE		Ds		Nano-objects		CIT_g (8min)	
CONC-A	-		67.42		-		0.009	
CONC-C	-		243.96	⇧	n.d.		0.063	
MORTAR-A	-		77.92		-		0.014	
MORTAR-C	-		31.37	⇩	n.d.		0.012	
FRPANEL-Control	191.68		1094.16		-		0.338	
FRPANEL-1.25%Dellite	154.85	⇩	1320		Evidences[1]		0.887	⇧
FOAM-Control	164.15		210.81		-		0.131	
FOAM-0.5%NCC	124.24	⇩	188.15	⇩	n.d.		0.134	
COM-Control	286.16		1320		-		0.750	
COM-0.5%CNF	296.27		1320		n.d.		0.637	

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P6-2

TECHNOLOGIES TO SIMULATE THE RELEASE OF ENGINEERED NANOMATERIALS (ENMs) FROM POLYMERIC NANOCOMPOSITES DUE TO MECHANICAL PROCESSES

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The main objective of the SIRENA LIFE project (Simulation of the Release of Nanomaterials from consumer products for environmental Exposure Assessment- LIFE+ ENV/ES/596) is to demonstrate and validate a methodology to simulate the unintended release of ENMs from consumer products by replicating different life cycle scenarios to be adopted by a variety of industrial sectors in order to get the necessary information for the exposure assessment.

In this framework, SIRENA has set up a Technological Surveillance System (TSS) to trace technical references related to the release of nanomaterials from nanocomposites. Out of the 73 items classified nowadays under "Nanomaterials release simulation technologies", 32 are specifically related to the degradation by mechanical processes: sanding, drilling, abrasion, cutting, sawing, grinding and shredding. For each of these references, relevant data have been extracted and summarized in a short sheet.

Identified references have been thoroughly analyzed in order to assess the state of the art in current technologies and protocols for the simulation of the release of nanoparticles from nanocomposites due to mechanical degradation. The aim is to assess their utility, applicability and reliability and to identify possible gaps.

After analyzing them, the most relevant outcomes have been:

- Due to the fact that no validated methodologies exist for scenario simulation, different groups apply different protocols.
- The absence of standard operating protocols to be used for scenarios simulation makes inter-assays comparison highly challenging.
- The majority of the items reviewed have assessed laboratory- scaled manufactured materials and in only a few cases the commercial reference of the nanocomposite material is provided.
- Generally, the application of the material tested is not described.
- In most cases, samples used as a reference (when used) are the same matrixes without the nano-scaled reinforcement.
- Most authors do not characterize the properties that the engineered nanomaterials confer to the test samples.
- Some authors have simulated the release in confined conditions (chamber, specifically designed enclosures, globe boxes…) whereas other authors have used open systems. In general, the lack of details provided prevents the replication of the experiments conducted.
- The instrumentation used is highly variable. The Scanning Mobility Particle Sizer (SMPS) or the Fast Mobility Particle Sizer (FMPS) in combination with Aerodynamic Particle Sizer (APS) and the Condensation Particle Counter (CPS) are some of the most frequently used instruments.
- There is no clear evidence on the differences of the emissions from the mechanical degradation of nanocomposites containing ENMs from the mechanical degradation of non-reinforced composites: no conclusions can be extracted from the existing data about how emissions vary with material composition.

The mechanical process and associated energy that is transferred to the samples has a direct effect on the nature and the quantity of emitted nano-objectsInformation about all the references analyzed in the project has been compiled in an online database which is freely accessible in a specific section on the website of the project (http://www.life-sirena.com). The information included is updated on a three monthly basis.

The SIRENA project (January 2013-December 2015) is co-financed by the Life+ Environment Policy and Governance (LIFE11 ENV/ES/596).

P6-3

NANOPARTICLE RELEASE QUANTIFICATION DURING LOW AND HIGH ENERGETIC DRY DISPERSING OF NANOSTRUCTURED POWDERS

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The basis for the fabrication of numerous nano-products are nanostructured powders. They were thus the subject of several nanoparticle release investigations (Kuhlbusch et al., 2011). Performed release studies can roughly be classified according to the applied dispersing stress into three treatment procedures: drop down procedures (e.g. Jensen et al., 2009), fluidized bed methods (e.g. Ogura et Al., 2009) and high energetic dispersing (e.g. Stahlmecke et al., 2009).
High and low-energetic dry dispersing procedures were used for powder aerosolization to estimate the quantity range of particle release into air from nanostructured powders according to ISO/TS 12025:2012. The former one based on the dispersing process within a rotating brush generator (Model RBG 1000, Palas GmbH, Germany) operated in combination with dispersion cover A at a dispersing pressure of 3.5 bar (relative flow velocity of 100 $m \cdot s^{-1}$), a volumetric powder flow of 25.7 $mm^3 \cdot min^{-1}$ and a rotational steel brush frequency of 1210 min^{-1}. In the absence of suitable commercial aerosol generators that produce steady-state particle number concentrations and particle size distributions from various powders at weak dispersing energy input, a dispersing device based on a linear-driven notch and a stainless steel capillary tube was developed (Göhler et al., 2010) and operated at a volumetric powder flow rate of 30 $mm^3 \cdot min^{-1}$ and a relative flow velocity of 24.4 $m \cdot s^{-1}$.
To characterize the simulated release scenarios, data were determined for the nanoscale, the submicrometre and micrometre size range using a scanning mobility particle sizer (SMPS, Model 3934, TSI Inc., USA) and an aerodynamic particle sizer (APS, Model 3321, TSI Inc., USA). The presentation will give information on the experimental setups, the performed evaluation of the measurement data and results of different nanostructured powders.

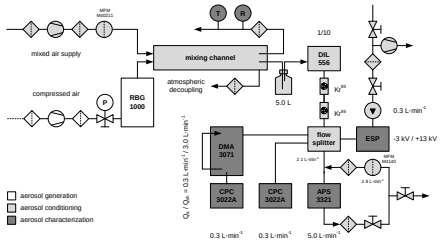

Figure 1. Schematic diagram of the experimental setup used for high energetic dry powder dispersion.

Acknowledgment.
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P6-4

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 NANOFlueGas has been dedicated to nanosafety aspects during the final destruction of nanomanufactured products by specialized thermal treatment. The main objectives of this project 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. Results obtained for objective 1 are presented here.
The selection and sampling of three types of hazardous wastes was performed on the basis of a preliminary identification of waste deposits potentially containing nano-contents. These wastes have different physical forms: a carbon residue (finely divided solid), an organo-silicon residue (viscoelastic solid), and a residue of water-based paints (liquid). The presence of nano-fillers in these residues has been shown and described qualitatively as well as quantitatively via an original multidisciplinary, analytical approach.
An experimental setup for incineration (primary chamber) has been selected, developed and fully instrumented for the measurement of gas and particulate phases. Such an experimental setup allows to reproduce at best at the laboratory scale, the conditions and Best Practices implemented industrially. The reproducible results showed a presence of nanoparticles in the primary incineration chamber during normal operation (excluding start-up and shutdown) in varying amounts, depending upon the tested waste type. The particle size distributions, during the incineration phase, are dominated by the size component <100 nm. The paint and polymer wastes generate abundant aerosol which consists mainly of nano-silica, while the powdery waste, much less contributory itself, mainly emits carbon nanoparticles.
Reference:
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, T. Meunier, O. Le Bihan, L. Le Coq, *nanosafe12*.

P6-5

BEHAVIOR AND FATE OF HALLOYSITE NANOTUBES (HNT_S) WHEN INCINERATING PA6/HNTS NANOCOMPOSITE

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Since the end of the nineties, nanoclays based nanocomposites have been widely studied and produced. These trendy materials are expected to end up in incineration waste plants. Recently, various studies have focused on the particulate emissions of polymer nanocomposites during their combustion [1, 2, 3]. The aim of our work is to investigate the behavior and the fate of the nano-objects from nanocomposites during their incineration. This study focuses on PA6/HNTs nanocomposites (polyamide 6 incorporating halloysite nanotubes). Incineration tests have been performed at lab-scale using a peculiar tubular furnace modified in order to control the key incineration parameters within both the combustion and post-combustion zones [4]. The combustion residues and the combustion aerosol (particle matter and gas phase) collected downstream the incinerator furnace have been characterized using various techniques devoted to the analysis of aerosols. Time tracking for gas concentration and particle number concentration reveals a two step mechanism. HNTs have been found both in aerosol and residues. As shown in Fig.1, some remains intact, while others are aggregated.

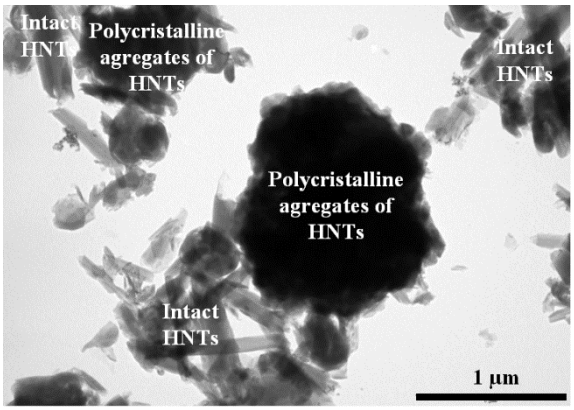

Fig. 1. MET Image of combustion residues with intact HNTs and polycrystalline aggregates of HNTs

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Authors thank: TREDI - Séché Environnement and Mines-ParisTech for their collaboration and ADEME for the financial support.
Keywords: combustion, incineration, nanocomposite, HNTs, PA6, aerosol, nanoclays

P6-6

STUDY OF NANOPARTICLES DUE TO THE EMISSION OF POLYURETHANE FOAM IN REAL CONDITION OF USE

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The CEA - NanoSafety Plateform develops various research topics for health and safety, environment and nanoparticles exposure, in professional activities. In its new laboratories, the PNS research teams met a high and unusual background. The concentration was unsteady and could exceed 100,000p/cm^3 for the maximum peaks. Several possible causes were explored before the discovery of the source of this high signal: some new chairs with polyurethane (PU) foam (cf. figure1). The hypothesis was that vapours of carboneous compounds are emitted by the foam. These vapours could condense by nucleation to form nanoparticles.

The contamination group of CEA-LETI is specialized in research and characterization of pollutant in the microelectronics labs. It performed various analysis by Thermal Desorption and Gas Chromatography-Mass Spectrometer (TD GCMS) on a sample of the foam, which mainly shown the emission of N,N,N',N'-tetramethyl-1,3-propanediamine and of other compounds in lower quantities. This molecule is known to be used for the polymerisation of the PU. The supplier confirmed that the chairs were made of PU.

In order to verify if the signal was really due to this molecule, 28 chairs were gathered in an empty room with a low air background. Measurements of airborne organic compounds and particles were organized respectively with a Tenax sorbent tube sampling followed by TD GCMS analysis and a Fast Mobility Particle Sizer (FMPS) of the PNS. The FMPS detected particles from around 10nm to about 40nm, with a maximum concentration around 80,000p/cm^3 like shown in figure 2. The comparison with a blank performed in the same room confirmed a gap of particles concentration without any significant difference in the chemical species sampled. Further experiments are ongoing.

Figure 6 : chair made of polyurethane

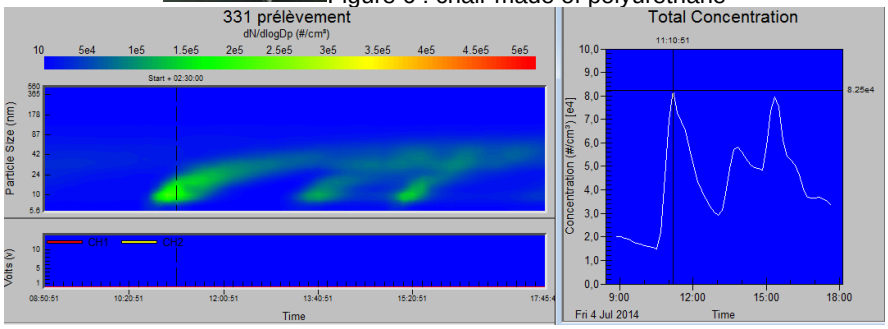

Figure 7 : Evolution of the concentration (right) and of the size distribution (left) during the sampling

P6-7

DUSTINESS OF BULK NANOMATERIAL POWDERS USING THE VORTEX SHAKER METHOD

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Bulk nanomaterial (NM) powders are increasingly being used worldwide in many industrial or research applications, and the number of workers and researchers involved in their handling and transport is thus growing. The release of particles from bulk NM powders into surrounding air is an important consideration as well for worker exposure as for the design and operation of many industrial or research processes.
Research conducted into the “dustiness” of powders with relevance to occupational exposure assessment has led to different methods that all involve the application of a specified type and amount of mechanical energy to a given amount of preconditioned powder and duration, whereby airborne dust particles are generated to be quantified. Besides the operation procedures themselves, the manner in which the output data is analyzed and reported is of importance.
These last years, studies have been performed in adapting the two methods described in the EN 15051 standard for NM powders (i.e. the rotating drum and the continuous drop systems), while new approaches have also been proposed based on a small rotating drum and a vortex shaker (VS) method.
Based on the original concept from Baron et al. (2002), the VS method developed within this work consists of a specially designed stainless steel tube that is continuously shook according a circular orbital motion (orbit 4 mm, rotation speed 2000 rpm), and in which a small volume (0.5 cm^3) of the test powdered NM is placed. HEPA filtered air, controlled at 50% RH, pass through the tube in order to transfer the released aerosol inside the tube to the sampling and measurement section. The current procedure is based on the use of: 1) a respirable selection before any aerosol measurement/sampling, 2) a CPC as reference instrument for number concentration measurement, 3) the MiniParticle Sampler (MPS, Ecomesure, France) as TEM grid holder to collect particles for EM observations/analysis, 4) the ELPI (normal or +, Dekati, Finland) as a size-resolved aerosol measurement technique. A two step approach has been defined to determine two dustiness indices: the respirable dustiness mass fraction (in mg/kg) and the respirable dustiness number fraction (1/mg). The VS method was evaluated in the dustiness investigation of 25 bulk NM powders that include high aspect ratio nanoparticles, metal oxides and miscellaneous NM. Moreover, the repeatability obtained after disassembly and reassembly of the test bench and by two different operators has been determined over 8 NM powders.
The results show that dustiness, quantified either by particle number or by mass-based dustiness indices, had a large range, over more than three orders of magnitude. These suggest a corresponding large difference in terms of potential exposure.
The VS method as proposed in this work together with the three others mentioned above are currently the subject a pre-normative research project (Dustinano) launched under the mandate 461 between the European Commission and CEN in order to develop a harmonized approach for evaluating dustiness for bulk NM taking into account the different existing concepts and test methods.

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P7-1

RECOMMENDATIONS FOR A NANOSAFE PRODUCTION OF NANO-DEVICE INVOLVED IN INFLAMMATORY DISORDERS TREATMENT

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NANOFOL is an European Project supported through the Seventh Framework Programme for Research and Technological Development. The project NANOFOL aimed to improve the treatment of chronic inflammatory diseases by the development and production of nanobiodevices (i.e. liposomes) in nanosafe conditions. As a result, a part of the Nanofol project is devoted to nanosafety management too with an aim of describing different operations which are used to deal with nano-risk during the use of dispersions containing nanoparticles. In the present communication, two parts of this work are discussed: the measurement campaign and the specific recommendations on nanosafety at a production site.

A classical method to perform a complete measurement campaign consists of a preliminary campaign with a particle counter and a particle sampler. If necessary, it may be accompanied by a complementary campaign. This measurement strategy is described in INRS-CEA-INERIS guide [INERIS-INRS-CEA-, 2012]. One of the applications of this kind of methodology is to verify the absence of the nano-suspension aerosolization in the ambience through an accidental leakage which may occur during the production of the nano-suspension under pressure [Nogueira, 2013]. The preliminary campaign aims to check such leakage. To do so, two analyses, counting measurement and TEM grid sampling [R'mili, 2013] respectively, are carried out. While the counting measurement assesses an emission, the TEM grid sampling allows the morphological /chemical characterization of aerosol particles.

The specific aspect of the recommendation part has to take into account percutaneous absorption risk induced by the liposome dispersion. For example Groso et al highlights the dispersant influence which facilitates the nanomaterials exposure [Groso, 2013].

It is possible to improve the nanosafety of the production site significantly by applying some modifications like carrying out each production step under a fume hood, particularly the filtration; purification of liposomes; implementation of double containment during each transport; labelled storage of nanomaterials etc. A proposition to simplify the nano-waste management has also been made. The producer could use such method to deal with their nano-waste more efficiently.

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Groso, A. and Meyer, T., Concerns related to Safety Management of Engineered Nanomaterials in research environment, 2013 J. Phys.: Conf. Ser. 429 012065.

P7-2

SAFETY BY MOLECULAR DESIGN: NANO CuO AS CASE STUDY

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In replacement to administrative, engineering and personnel protective equipment (PPE) based control measurements, (as for instance local exhaust ventilation using HEPA filters), new preventive remediation strategies have the chance to design out the risks rather than address them when occur. CuO commercial nanopowder (PlasmaChem GmbH) used as active component in the formulation of antimicrobial (preserving) wood coating, has been selected as case study. Understanding how surface engineering modifications affect colloidal properties in water is fundamental to predict and drive nanomaterials fate and kinetics in more complex media, as well as some biological relevant doses such as the total content and the bioavailability of metal ion fraction. The results provide necessary information for the control of biological reactivity in a Safety by molecular Design approach (SbyD). The following trends have been observed:

- Trend vs pH dependent surface charges. (1);
- Trend vs surface adsorbed moieties. Three surface capping agents, differing for their charge (negative, neutral, positive) were added and let mixing with nanoparticles water suspension in order to promote the creation of self-assembled layers of additives on particle surface (2).

Every experiment has been followed by characterization of the gained colloidal stability, expressed by Zeta potential determination, mean particle size, aggregation factor and fraction of ion copper dissolved.
Results showed that copper oxides nanopowder without any stabilizing agent produce suspensions that are only slightly stable in the pH region close to neutrality, at basic pH these exhibit strong aggregation and subsequent precipitation, leading to poor transport fate mechanism and giving pollution of soils and sludge instead of superficial waters. On the other hand, acid solutions induce copper dissolution and therefore it is expected that the prevailing toxicity come from the dissolved fraction of Cu^{2+}. Titration curves reporting Zeta potential as a function of amount of added capping agent let to identify around 10 weight % the minimal amount of capping agent necessary to coat the powder. Samples coated with different capping agent and buffered at basic pH were prepared and the relevant colloidal properties assessed. Aggregation factors were calculated by comparing DLS size distribution with BET diameter, revealing a high tendency of powder to aggregate in water medium, not mitigated by the presence of coating agents and easly detected by SEM-FEG observation. Finally a correlation between type of capping agent and corresponding Cu^{2+} / CuO weight ratio was found.

The research leading to this commentary has received funding through the project “SUN” (NMP4-LA-2013-604305)

P7-3

EFFECTIVENESS OF N95 DISPOSABLE PARTICULATE RESPIRATORS AND FPP3 HALF MASK RESPIRATORS AGAINST TARGET NMS FOR THE PIGMENT AND INKS INDUSTRY

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In the particular case of the pigment, ink and paint industry, the use of engineered nanoparticles (ENPs), have a great potential for new applications, leading to products with new or enhanced properties, and opening new market opportunities. Consequently, many promising applications emerge nowadays, based on the use of ENPs such as Fe3O4, TiO2 or ZnO or quantum dots (QDs). Along with the benefits, there is an on-going debate about their potential effects on human health or the environment, considering as a key issue the potential adverse effects of ENPs on workers upon inhalation. In this sense, it has been demonstrated that ENPs can become airborne during common industrial activities, some of them related with the production of nano-pigments and/or nano-inks. These airborne particles, including nanoparticles and ultrafine particles may enter into the human respiratory tract via inhalation.

Considering the growing production of nanoparticles to develop high-tech applications, there is an urgent need to define adequate risk management measures to mitigate and control the exposure. A first step to protect workers is to enhance the knowledge on the effectiveness of current risk management measures, including personal protective equipment (PPE) and engineering controls (OC).

A complete evaluation of the effectiveness of common RMMs against ENPs at the workplace has being carried out under the scope of the FP7 project NanoMICEX (NMP4-SL-2012-280713). We present here the results encountered during the evalaution of the protection factor (APF) and leakage efficacy effectivness of two different types of respirators, including a N95 Disposable Particulate Respirator and a FPP 3 reusable half mask respirator. The selected RMMs were evaluated in the testing chamber designed and developed by ITENE. A picture of the testing chamber and the Sheffield head employed during the test are depicted below:

Fig. 1. Sheffield head (Courtesy of the INSHT) and the Aerosol Testing Chamber developed by ITENE

The experimental protection factor was defined by the ratio between the number concentration of particles upside the protective device (Cupstream) and the concentration within that device (Cdownstream). The concentrations upstream were measured by means of a Philips Nanotracer, which detects particles below 300 nm and an Optical Particle Sizer (OPS-TSI), which detects particles up to 10 microns. Downstream the particles where detected by means of a CPS System (CPC model 9007 – TSI). To conduct the tests with our target ENPs, it was necessary to use a powder aerosolizer, in this case, the Naneum powder aerolizer PA100.

Average penetration levels for the two different masks were between 26 and 2%., with a minimum penetration level for the Reusable Half Mask respirator. The results showed significative differences in the penetration factor for the models studied. It shall be noted also that the PF characterized are higher than the recommended 5%, which means that none of the mask tested are effective enough against ENPs

P8

HUMAN TOXICITY AND FRESHWATER ECOTOXICITY CHARACTERISATION FACTORS FOR ENGINEERED NANOPARTICLES: TOWARD A SPATIAL DIFFERENTATION

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Engineered nanoparticles (ENPs) are increasingly used in several applications, from medicine to environmental remediation. In the last decade several concerns on their potential human and environmental impacts have been raised. ENPs release into the environment may occur throughout their entire life cycle: from production to the fabrication of engineered nanomaterials (ENMs) containing ENPs, to the use and end of life phase of those products. Hence, Life Cycle Assessment (LCA) has been addressed as a systematic method to determine the human and environmental impacts of nanotechnology products at several stages of their life cycle. In spite of this only a few LCA studies on nanotechnology and on ENPs have been published to date and, even fewer assessed the human and ecotoxicological impacts. Currently, the knowledge gaps in the field of risk assessment of nanotechnology are reflected in LCA, where characterisation factors (CFs) for toxic impact categories are missing. The determination of characterisation factors requires the knowledge of the environmental fate of a substance, human and environmental exposure and its toxicity. Within the Life Cycle Impact Assessment (LCIA) for the no-toxic impact categories (e.g. Climate change), no special difficulties in the assessment of nanoproducts can be foreseen. On the other hand, for toxic impact categories, the current knowledge on the toxicity of ENPs and on their environmental fate, exposure and toxicity may not be sufficient for a representative characterization of nanoproducts. The present research is focused on LCIA with the aim of presenting the possibilities of CF calculation for metal oxide nanoparticles (as n-TiO_2), for the impact categories of human toxicity and freshwater ecotoxicity and considering spatial differentiation. The framework proposed is based on the USEtox model. Morover, it applies the recent multimedia environmental model (SimpleBox4Nano) developed by Meester et al. 2014 to calculate the fate factor for n-TiO_2 considering both air and freshwater comparents. Thus, specific-fate process for ENPs (attachment, aggregation, dry deposition ect.) have been accounted and described by first order kinetic rate. Whereas, the human and environmental exposure have been calculated following the method proposed by USEtox framework. The fate and exposure factors to ENPs are evaluated accounting several geographical scale (continental, urbans) and spatial archetypes (Sala et al., 2012). In fact within the fate calculation the systemic dimensions (e.g *area, height, volume of atmosphere, soil and water)*) and (*ENPs radius, ENPs mass density, aggregation efficienty and attachement efficienty*) have been assessed to different spatial scale. Hence, the present study allows to calculate the CFs for human toxicity and freshwater ecotoxicity for n-TIO_2 accounting for different spatial scale.

P9-1

DEVELOPMENT OF A TECHNICAL SPECIFICATION: GUIDELINES FOR THE MANAGEMENT AND DISPOSAL OF WASTE FROM THE MANUFACTURING AND PROCESSING OF MANUFACTURED NANO-OBJECTS

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Mandate M/461 is a request for standardization activities regarding nanotechnologies and nanomaterial addressed to CEN (European Committee for Standardization), CENELEC (European Committee for Electrotechnical Standardization) and ETSI (European Telecommunications Standards Institute) by the European Commission.

In 2010, CEN accepted 'Mandate M/461 - Standardisation Mandate to CEN, CENELEC and ETSI for standardisation activities regarding nanotechnologies and nanomaterials'.

Mandate M/461 identifies four areas for standards development:

- Methodologies for nanomaterial characterization in the manufactured form and before toxicity and eco-toxicity testing;
- Sampling and measurement of workplace, consumer and environment exposure
- Methods to simulate exposures to nanomaterials
- H, S & E (health, safety and the environment)

Since 2013, the CEN Technical Committee CEN/TC 352 'Nanotechnologies' is involved together with CEN/TC 195 'Air filters for general air cleaning' and CEN/TC 137 'Assessment of workplace exposure to chemical and biological agents' in developing several European deliverables under this Mandate.

Under this mandate, a CEN Technical Specification (TS) “Guidelines for the management and disposal of waste from the manufacturing and processing of manufactured nano-objects” is being developed by CEN/TC 352 WG3 /PG4 ‘Waste’.

This TS will provide guidance for all waste management activities from the manufacturing and processing of manufactured nano-objects. This guidance will be of use to manufacturers and waste disposal companies. However, it is not intended to provide guidance on the management and disposal of nanocomposites, waste derived from consumer products containing nano-objects or waste containing only naturally occurring and/or incidental nano-objects. Neither, will this TS aim to give guidance on the management and disposal of non-nanomaterials waste derived from the manufacturing and processing of manufactured nano-objects.

This poster will raise awareness of the current development of this technical specification and to aid consultation with any interested parties.

This work is funded by the European Commission and EFTA and managed by AFNOR holding the Secretariat.

P9-2

GROUPING OF NANOMATERIAL BY HEALTH, SAFETY & ENVIRONMENTAL CHARACTERISTICS

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In the nanoGRAVUR project several manufactures, distributor, research institutes and regulators will elaborate assessment criteria for the risk identification and evaluation of nanomaterial hazards for human and environment.

15 partners will examine 40 nanomaterials and formulations upon the possibility to describe standard characteristics, emission and exposure patterns, hazardous and toxicological properties, measurement strategies, waste management as well as risk management measures.

One of the partners is IFA - the Institute for Occupational Safety and Health of the German Social Accident Insurance. IFA´s interest in the project is to find harmonized assessment criteria for workplaces where nanomaterial is handled. In nanoGRAVUR next to a literature study material characteristics will be tested in experiments and exposure pattern simulated in laboratory as well as field tests.

The findings within the project could serve as a harmonized basis for the more specific risk assessment of workers exposure to nanomaterial on the one hand (picture 1) and decision support criteria for legislators, regulators, inspectors and supervisors on the other hand.

Picture 1: Assessment of characteristics, exposure, toxicology and ecotoxicology factors should lead to generic descriptors for nanomaterial hazards

During the project findings will be exchanged and discussed for their validity and practicability not only between the certain working groups but also evaluate d by several external experts and stakeholders in interdisciplinary workshops.

P9-3

CEN/TC 352/WG3/PG3 – "PROTOCOLS FOR DETERMINING THE EXPLOSIVITY AND FLAMMABILITY OF POWDERS CONTAINING NANO-OBJECTS (FOR TRANSPORT, HANDLING AND STORAGE)"

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In order to promote sustainable development of nanotechnologies, standardisation has to be considered as a pillar for success. Indeed, the current lack of standardisation allowed tensions to increase between the various stakeholders (researchers, industry, public authorities and consumers) as it entails the jeopardization of both commercial and regulatory perspectives. Thus, when considering standardization, safety appears very quickly as an outstanding and essential requirement. New powders containing nano-objects imply new 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 powders containing nano-objects, especially their propensity to burn, explode and disperse in case of a loss of containment, in order to manage industrial risks. The explosivity and flammability properties have to be given in the safety data sheet for a safe storage, handling and transport of any powder and are key safety parameters to ensure proper industrial safety management.

In this perspective, the European Committee for Standardization (CEN) set up the Technical Committee 352 (CEN/TC 352) in 2006 to develop and maintain up to date standards in the field of nanotechnologies. Part of the work group (WG 3) dedicated to Health, Safety and Environment, the CEN/TC 352/WG3/PG3 led by INERIS was constituted to develop a Technical Specification (TS) for the determination of explosivity and flammability properties of manufactured nano-objects in powder form.

Since its establishment in January 2013, the group has set milestones to complete its objective:

- The review and analysis of categorization modes of existing nanomaterials, in view of the latest standardization and regulatory initiative at European and international level in order to underline relevant metrology to assess safety parameters (for explosion sensitivity and severity and for flammability).
- A critical review of current limitations of apparatuses and methods as knowledge on nanoscale safety parameters tends to differ from those at macroscale.
- The development of new tools and methodologies in the aim of getting reliable characterization of physico-chemical hazardous properties of nanomaterials. Indeed, existing tools and test procedures (such as the 20 L explosion sphere, the modified Hartmann tube, etc…) designed to evaluate dust explosion hazards may have to be modified and tested to handle nanoparticles specificities.
- The organization of interlaboratory tests to validate those potentially new experimental devices and protocols.
- The elaboration of the Technical Specification protocols for determining explosivity and flammability of nano-objects by 2018.

These investigations will be supported by several experts, part of reference institutes in the field and with an acknowledge expertise in explosivity and flammability.

This project is still in its early days as its work will be complete by the second half of 2018. The broad panel of experts from standards organizations, industry and public authorities (France, Germany, Great-Britain, Belgium, Spain, Czech Republic…) will enable to develop dedicated protocols that will be acknowledged by the various stakeholders involved in the development of nanomaterials.

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P9-4

REFERENCE NANOPARTICLES OR REFERENCE PROTOCOL: WHAT SHOULD COME FIRST TO MAKE SIGNIFICANT PROGRESS IN HEALTH RISK ASSESSMENT?

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Nanomaterials are widely present in many industrial sectors (e.g., chemical, biomedical, environment), and their applications are expected to significantly improve in the coming years. However, the use of nanomaterials still raises many questions regarding potential risks to human health, the environment, and more specifically, occupational health. During the past decade, the scientific literature has been significantly improved due to a higher systematic process of the chemical characterization methodology of the nanoparticles used in the experiment. However, still in 2014, several key questions regarding dose metrics, nanomaterial characterization, analytical methods, reference materials, reference methods, harmonized protocols, among others remain unanswered. Harmonized and reference-based approaches are essential for health risk assessment because they provide opportunities to compare published studies, thereby improving the accuracy of the choices that the regulators need to take to protect human health. This is of particular concern for nanomaterials when inconsistent data are reported when, for example, multi-walled carbon nanotubes from two companies express widely different toxicity. Such issues can be addressed in scientific papers, but remain the authors' opinion, based on what they believe. Another way to address these issues is to organize a scientific debate where experts can discuss—and hopefully reach consensus—about what they believe should be accepted as a reference. *To this end, a scientific debate will be organized at Nanosafe 2014 to discuss what should come first—the reference nanoparticles, the reference protocol, or both in parallel—and then respond to different questions related to this main question*. The debate will involve a panel discussion with four experts who will discuss their ideas about specific questions prepared by the panel's coordinator. Each question will be followed by a short response from each expert. The attendees of the debate will also be given opportunities to discuss the topics and make comments. The goal of the debate is to ask four specific questions about references and prioritization topics, and then try to reach consensus among the panel members and the audience, regarding the main question. The debate will be summarized in a report to be published in the workshop proceedings.

P11-1

HUMAN RISK ASSESSMENT AND ITS APPLICATION TO NANOTECHNOLOGY: A CHALLENGE FOR THE ASSESSOR

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Scientific literature suggests that exposure to nanoparticles (NPs) might be associated with adverse health effects (Emond 2011; Fujita *et al.* 2014; Matthews *et al.* 2013; Tian *et al.* 2013). However, a well-developed human risk assessment (HRA) that applies to NPs has never been established and optimized until now. **In addition, there are no government regulations in place that establish what is considered to be an adequate and secure level of exposure and supported by a strong scientific approach for nanotechnology.** It is mandatory to implement the HRA to ensure that workers producing NPs, users of NPs, and the general population are protected from deleterious issues related to NPs. This methodology was initially described in a book (commonly referred to as the "Red Book") published by the National Research Council (NRC) in 1983. This book classically divided the HRA into four different characterization steps: source identification characterization (SIC), exposure assessment characterization (EAC), hazard assessment characterization (HAC), and risk assessment characterization (RAC) **(NRC 1983; NRC 1994).** The aim of this work is to review the different characterization steps of the HRA and to discuss the current limitations that apply to them regarding nanotechnology. The SIC represents a straightforward step regarding the occupational point of view because it is relatively well understood. Nevertheless, that may represent a challenge for the general population because NPs are found in many different consumer products. Without a labeling regulation in place, it is difficult to determine whether NPs are present in consumer products. The EAC represented a challenge for the metrology because existing equipment and instrumentation are not always adequate for NPs. The most problematic of the steps is certainly the HAC because currently no consensus exists on many points of view such as the physical and chemical characterizations of the NPs, dosimetry, and toxicological reference protocols. Moreover, there is an important requirement to develop or identify toxicological protocols adapted to NPs. In fact, in an in vitro cellular assay, the same NPs in two different culture media can generate completely different toxicity profiles. In one case, the result reveals a toxicity, but in another, the finding shows a weak toxicity or no toxicity (Kroll et al. 2009). The RAC, which represents the combination of the HAC and the EAC, interprets the level of risk based on the exposure level affecting the exposed workers or the general population and the level of HAC, corresponding to the sensitivity of the NPs to react with biological tissue. This step represents a serious problem for risk assessors because there are several limitations in EAC and HAC that result in missing data. **Some work has been initiated** by our group to fill the gap of nanosafety assessment by measuring the impact earlier in the process than when they appear on the market (Emond et al. 2013). Nanotechnology is meant to play an essential role in the development of today's modern societies. There are many great advantages associated with the use and application of NPs, but with these come serious risks to human health. In fact, greater effort is required during synthesis and before the commercialization phase to process a systematic and rigorous assessment that could significantly reduce the risks to workers and the population.

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P11-2

LIFE NANORISK – BEST PRACTICES, EFFECTIVENESS, PREVENTION AND PROTECTION MEASURES FOR RISK CONTROL POSED BY ENGINEERED NANOMATERIALS

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NanoRISK is a research project co-founded by the European Commission, through the LIFE+ Environmental Policy & Governance program. The project was launched in October 2013 and is expected to be completed in September 2016. The project is coordinated by ITENE and implemented in partnership with VITO, AVANZARE, CRP, INVASSAT, INSHT.

The main objective of the project is to define proven Risk Management Measures (RMMs) to prevent or minimize exposure to engineered nanomaterials (ENMs) during specific workplace situations, as well as to support standardization activities concerning the certification of the adequacy of Personal Protective Equipment (PPE) and Engineering Controls (ECs) to protect workers from the risk posed by the use of ENMs.

The specific project objectives are:

- To define proven RMMs for mitigating and control the environmental, health and safety (EHS) risks posed by ENMs.
- To support the Library on RMM (RMM library) developed within the REACH Implementation Projects with quantified data on the effectiveness of personal protective equipment (PPE), engineering techniques and organizational measures.
- To develop a test chamber prototype to evaluate and demonstrate the performance of the RMM at laboratory scale.
- To define a compendium of standardized methods based on international standards to evaluate the effectiveness of PPE and collective protection measures.
- To enhance the knowledge base on the releases of NMs to air, soil and water from industrial settings
- To improve the knowledge on the likely exposure scenarios in the nanocomposite industry.
- To support the hazard and exposure characterization for ENMs with the aim to support industry in carrying out their Chemical Safety Assessment (CSA) as stated by REACH.
- To disseminate the project results for a large community of SMEs and potential stakeholders.
- To support the monitoring of REACH compliment and its impact on risk mitigation and prevention of pollution posed by NMs.

The main outcomes of the project will be a library of proven and technically feasible prevention and protection measures for mitigating and controlling the environmental, health and safety (EHS) risks posed by nanomaterials during production, use and release, as well as a set of standardized testing protocols based on the application of a newly designed test chamber to support the quantitative evaluation of the effectiveness of workplace controls:

We will present the developed test chamber and testing protocols at NanoSafe 2014.

Acknowledgement: The NanoRisk project is supported by EU LIFE programme (LIFE12 ENV/ES/000178)

P11-3

REACHnano Tool: A NEW WEB BASED TOOLKIT TO SUPPORT THE CHEMICAL SAFETY ASSESSMENT OF NANOMATERIALS (ARIAL 10, BOLD)

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REACHnano is a LIFE + project (LIFE11 ENV/ES/549) focused on the development of innovative instruments to improve the implementation of the European Union Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) when manufacturing or handling materials or substances at the nanometer scale. To this end, the project was structured to allow the **development of a web based Help Desk tool to support the risk assessment and promote the safety use of nanomaterials along their life cycle**, providing the industry and stakeholders with easy to use tools to support the implementation of REACH regulation.
The toolkit developed within REACHnano project takes into account the needs and specifications of end-users and stakeholders, including advanced functionalities that supports the industry and authorities to fulfill their main task under REACH, with special concern to those provisions aimed at ensuring high levels of human health and environmental protection such as the generation of reliable information in terms of REACH information requirements , the assessment of risk for the specific uses of the substances (i.e. exposure scenarios) and the characterization of effective risk managements measures (RMMs).
The main contents of the web based tookit are a ENMs database module, the risk assessment plug-in and the advanced query tool. The design of the toolkit was done in collaboration with the partners of the consortium, including LEITAT Technological Centre, NIA - Nanotechnology Industries Association, and INVASSAT. Figure 1 illustrates the toolkit front end.

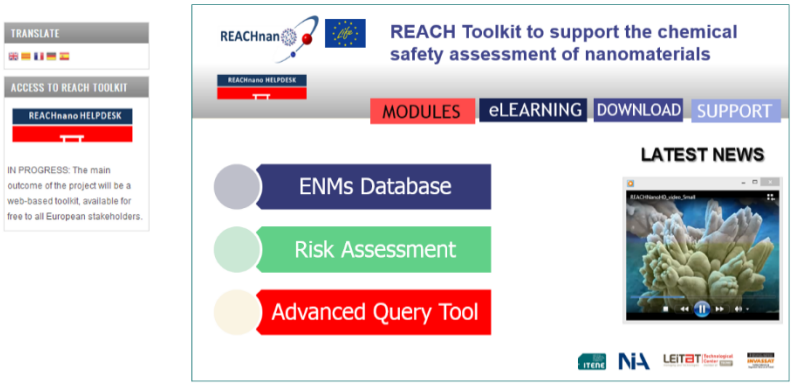

Figure 1. REACHnano Toolkit Front-end

The ENMs database has been designed following the structure of the IUCLID substance datasheets, allowing companies and relevant stakeholders to capture, store, submit and exchange data. The advanced query tool, it is aimed to serve as an innovative web-based data mining tool to support the identification of safer alternatives to hazardous nanomaterials.
The risk assessment module consists of two risk assessment plug-ins for occupational and environmental exposure respectively. The Environmental exposure plug-in is a probabilistic Material Flow Analysis (pMFA) multi-media model based on Monte Carlo (MC) methodology, while the occupational risk assessment module is based on a combination of control banding approaches , exposure estimation tools, and newly developed exposure scenario templates, allowing the uses to estimate the exposure on the basis of the operative conditions and RMMs applied in generic and/or specific exposure scenarios (GES / SES).

P12-1

REGULATION AND INNOVATION DYNAMICS FOR NANORESPONSIBLE DEVELOPMENT: THE CASE OF THE FRENCH CODE DE L'ENVIRONNEMENT, L 523-1 TO L 523-5

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The objective of the paper is to examine the impact of the French Code de l'environnement, Articles L 523-1 to L 523-5 on innovation dynamics. Articles L. 523-1 to L. 523-3 of the Environment code provide for the obligation to declare the quantities and uses of substances at nanoscale produced, distributed or imported in France. This procedure is intended to improve the knowledge of these substances and their uses, to ensure the traceability of sectors using these substances, to improve the knowledge of the market and the volumes sold, and to obtain available information on their toxicological and ecotoxicological characteristics. The paper builds on recent work on the emergence of a regulatory framework for nanotechnologies to take stock of the current situation in France, in the EU and globally and to explore how this specific law package may influence innovation and the shaping of new markets for nano-based materials.

Our methodology rests mostly on archival work, completed by some focused interviews. After analysing a comparative database of existing regulatory frameworks both inside and outside the EU, we study the evolution of where CAC 40 firms locate their R&D centres globally. The CAC 40 (Cotation Assistée en Continu) is a benchmark French stock market index which represents a capitalization-weighted measure of the 40 most significant values among the 100 highest market capitalizations on Euronext Paris. Euronext Paris is itself part of the Euronext Group, one of the major stock exchanges globally. Traditionally, the CAC 40 index represented the most powerful French companies and it is almost exclusively composed of French-domiciled firms. The link between innovation and R&D, as well as the importance of the geographical location of R&D in a globalized world have been documented in the literature (Gerybadze and Reger, 1999, Doz, 2012). Besides, nanotechnologies have been described as diffuse technologies that require integration to fulfil their economic promises (Avenel et al., 2007) and most key industrial players carry out nano-related activities in their R&D centres (Hernandez Guevara et al., 2013). We posit that if CAC 40 firms feel that French regulation has a negative impact on their freedom to innovate, either by restraining some fields or research, or by enforcing costly and restrictive compliance measures, they will opt to relocate R&D centres outside France.

Our study shows that nano-regulation does have an impact on innovation. However, the impact is not the same for EU regulation as for French regulation, and EU regulation seems in a way to boost innovation while French regulation stifles it. With this study we hope to bring new perspectives to the field of the strategic management of innovation, and also to shed some light on the roles and challenges of institutions to facilitate nanoresponsible development.

P12-2

THE DANA2.0 KNOWLEDGE BASE NANOMATERIALS – COMMUNICATING CURRENT NANOSAFETY RESEARCH

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The success of nanotechnology is particularly based on its versatility. It will bring about fundamental changes of basic research as well as of many sectors of industry. It will also have a great impact on our daily life ranging from electronics to the health care system. However, consumers often miss reliable and understandable information on nanomaterials and nanotechnology and don't know where to get such information.

There is a great need to respond to basic questions such as *"What exactly are nanoparticles?", "Are there any risks for myself and the environment?"* or *"What's the current research doing to solve these questions?* These and many more questions are answered by the DaNa$^{2.0}$ Knowledge Base Nanomaterials on www.nanopartikel.info.

Our international expert team brings together its expertise and knowledge from different research areas dealing with all aspects of nanosafety research in order to create and provide a non-biased, quality-approved and up-to-date knowledge base for more transparency.

The DaNa$^{2.0}$ project publishes articles covering latest research results on nanomaterials with regard to their influence on humans and the environment in an easily comprehensible way. The needs of different recipient groups such as interested laymen, stakeholders and scientists are addressed simultaneously by making use of a sophisticated article structure

For this purpose, scientific publications, reports, project results (funded by the German Federal Ministry of Education and Research) and latest news on human and environmental toxicology are analysed. The current state of knowledge is wrapped up in the knowledge base. The central tool of our integrated application-based database provides a unique link between nanomaterials in real applications (e.g. environmental remediation or medical products) and their potential impacts/ toxicological effect(s) and can be easily accessed by the interested visitor.

Using the «Literature Criteria Checklist», a customised methodology developed by the DaNa expert team, helps to discriminate between high- and low quality publications and thus facilitates the evaluation process of scientific publications. This checklist includes the definition of mandatory and desirable assessment criteria in accordance with quality criteria that have been acknowledged worldwide within the scientific community.

Additionally, DaNa$^{2.0}$ provides a list of FAQs, a link platform with contact data to other information portals and the opportunity to directly pose questions to our experts via E-mail. DaNa$^{2.0}$ is also present on Twitter, follow us @nano_info.

DaNa$^{2.0}$ is a German umbrella project funded by the German Federal Ministry of Education and Research (FKZ 03X0131) and is supported by Swiss Federal Authorities.

P12-3

NANOTECHNOLOGY REGULATION: MULTILATERAL INITIATIVES FOR A RESPONSIBLE AND BENEFICIAL DEVELOPMENT OF NANOPRODUCTS

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The nanotechnologies as a new technological and industrial revolution are creating a wealth of new materials, technological platforms, manufacturing and product performance enhancement, which profoundly impact our economy, our environment and our society. Nanotechnology-based products are available to consumers in many countries already, including Brazil. Additional products and applications are currently in the research and development stage, and some may reach the market soon. In view of such progress, it is expected that nanotechnologies-derived products will be increasingly available to consumers worldwide in the coming years, including overall food and health products. The potential benefits of the nanotechnologies are immense, but so are the potential dangers. In order to avert possible dangers, specialists and authorities should thoroughly understand them, and develop comprehensive plans to prevent potential risks, which include regulation aiming a beneficial and responsible development. Globally, in nanosafety aspects there is a very slow development. All the society segments claims for safer development as well as whole governance of the overall nanotechnologies. In this sense the Brazilian Health Surveillance Agency as well as similar health surveillance agencies, worldwide, are working on initiatives to understand and overcome the several safety gaps to contribute for a safe, responsible and beneficial nanotechnologies development. Based on the worldwide scientific findings: a) there are undersupplied investments in nanosafety; b) there are insufficient nanosafety investigations; c) there are not enough and well prepared scientists to meet the rising safety research demands of the new nanotechnological products; d) in general, the current international standards have to be improved for confident safety analysis; and e) There is an urgent need of capacity building for nanorisk analysis in the regulatory agencies. In this sense, in our perception, multilateral initiatives as well as a global coalition of regulatory agencies could speed up the development of solutions to overcome such safety and regulation gaps to contribute for a safe, responsible and beneficial nanotechnologies development.

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CLASS ACTION LITIGATION FOR SKIN CANCER BY SUNSCREENS

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In 2006, consumer lawsuits filed in Los Angeles Superior Court alleged manufacturers of sunscreens, including the popular Coppertone and Banana Boat brands, made false claims that exposed millions of innocent people to skin cancer. The suits were combined into a class action against Merck, the manufacturer of Coppertone, on the grounds Coppertone lacked the labeling to warn consumers about the dangers of skin cancer from prolonged sun exposure. Prior attempts by the FDA to require sunscreen manufacturers to provide similar warnings on their sunscreens were stayed by intense industry lobbying. Because of this, the class action sought to have Merck remove labels that Coppertone provided a “sunblock” and “all day protection” against harmful UVA and UVB rays known to cause skin cancer. Merck countered by arguing the Coppertone labeling and advertising were in compliance with all applicable laws and FDA regulations and further remarked the lawsuit was litigation gone amok as no consumer lost more than pocket money paid for the sunscreen. Since no one alleged medical damages, the Merck class action suit only sought the removal of misleading labels on Coppertone that the FDA could not do because of the lobbyist. In 2012, the Court ordered Merck to create a $10 million fund to cover monetary relief for millions of consumer claims, each claimant recovering at least the cost of Coppertone purchases. Also the Court ordered injunctive relief having Merck remove the terms "sunblock" and "all day protection" from Coppertone labeling.

The Court ruling excluded monetary relief for skin cancer by consumers, but allowed medical damage lawsuits to be brought in separate lawsuits based on the Merck precedent. In this regard, lawsuits alleging skin cancer caused by sunscreens containing nanoparticles have merit because for decades experiments have shown nanoparticles in body fluids cause DNA damage which, if not repaired by the immune system, leads to cancer.

Currently, the potential for zinc oxide ZnO nanoparticles commonly used in sunscreens to cause skin cancer is thought to depend primarily upon the ability of the nanoparticles on the skin surface to reach the epidermal layer. Recent experiments show human immune macrophage cells do remove ZnO nanoparticles from outer skin layer; however, DNA damage was not reported. But in another study, cobalt Co-chromium Cr nanoparticles placed on one side of a cellular barrier were found to damage the DNA of human fibroblasts on the other side, even though the nanoparticles never crossed the barrier, a finding that suggests DNA damage did occur with sunscreens even though the ZnO nanoparticles never reached the epidermal layer. .

At Nanosafe 2010, the author here proposed the toxicity of nanoparticles was caused by QED induced non-thermal EM radiation based on the QM argument that under TIR confinement the atoms in nanoparticles lack the heat capacity to conserve thermal energy from body fluids by an increase in temperature. QED stands for quantum electrodynamics, EM for electromagnetic, QM for quantum mechanics, and TIR for total internal reflection. By this theory, nanoparticles conserve thermal energy by the steady emission of EM radiation that at UV and higher frequencies does indeed damage the DNA at a distance. Although nanoparticles in sunscreens do absorb harmful UVA and UVB rays from the sun as claimed, QED converts the absorbed EM energy to far more damaging UVC radiation. Therefore nanoparticles in sunscreens make the DNA damage worse than if they were not used at all.

In this paper, a class action lawsuit following the Merck precedent is presented that requires the manufacturers of sunscreens to add labeling such as “contains nanoparticles known to damage DNA that may lead to skin cancer” thereby allowing the consumer to decide on whether or not to purchase the sunscreen.

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OMNT, A STRATEGIC WATCH ORGANIZATION

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Nowadays, nanomaterials are commonly found in a wide range of daily products, from food to cosmetics and toys. Through the threat of nanoparticles pollution and the use of nanotechnology in industry and medicine, a better understanding of nanomaterials becomes more and more essential. Towards this outcome, The Observatory of Micro and Nanotechnologies (OMNT), with the cooperation of more than 300 experts in the domain, aims to compel the day-to-day knowledge of nanomaterials technology and its use and safety.

Indeed, this joint unit between the CEA and CNRS, created in hope to associate different fields of expertise and therefore offer a better vision of the nanomaterials issues, includes 15 main topics, 3 of which are related to Life Science, such as the cluster "Environmental, Health and Safety impacts of Nanoparticles" (EHS group). In this group, experts from varied institutions, including the IRSN, ANSES, InVs or Cerege, discuss and debate in depth the state-of-art and the innovation outbreaks of their field, 4 to 5 times a year. This strategic watch is then summarized every 3-4 months in scientific reviews, available on our website www.biblio-omnt.fr (**fig.** 1A). Moreover, the OMNT produces every month a Newsletter related to Nanosafety (**fig.** 1B), distributed to a large Scientific and Industrial audience. More than just a strategic watch initiative, the OMNT has also successfully held occasional and recurrent events, organized focused group analysis and produced comprehensive studies. Furthermore, due to the importance of European legislation on nanoparticles and safety, the EHS group has always aimed to gather with European players in the field to expand the discussions and collaborations. Indeed, as part of the Nanosafety cluster, the EHS group had developed, between 2010 and 2012, a specific European Strategic Watch.

Thus, the OMNT offers a unique possibility to gather national and international experts in EHS, and welcome any new member who wants to share and discuss nanomaterials use and safety.

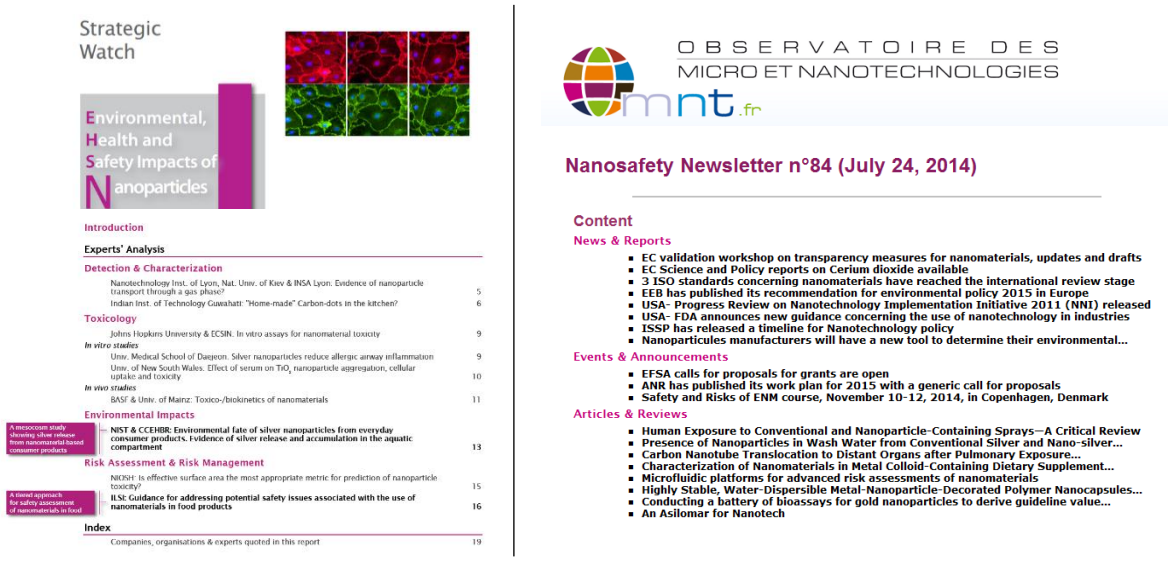

***Fig**. 1 Example of the Strategic watch document (A) and Nanosafety Newsletter (B) produced by the OMNT.*


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