'Nano in Belgium' Workshop

22 October 2015

8:30 – 17:45

Room Storck, FPS Employment, Labour and Social Dialogue

Ernest Blerotstreet 1

1070 Brussels

Book of abstracts

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Agenda

8:30 - 9:00 Registration

9:00 - 9:15 **Welcome by *R. Moreau*,** *General Director, DG Environment, FPS Health, Food Chain Safety and Environment*

Session 1: BE Nano-registry

Chair: ***C. Dantinne**, FPS Health, Food Chain Safety and Environment*

9:15 - 9:35 The Belgian national registry of nanomaterials (***C. Gorrebeeck,*** *FPS Health, Food Chain Safety and Environment*)

9:35 - 9:55 Workers' safety in the context of the Belgian Nano-registry (***L. Wouters,*** *FPS Employment, Labour and Social Dialogue*)

9:55 - 10:10 Q&As

10:10 - 10:25 **Coffee break**

10:25 - 10:45 Lessons learned from the French nano-registry (***V. Coissard**, Ministry of Ecology, Sustainable Development and Energy, France*)

10:45 - 11:05 Pilot exposure registration working with nanomaterials in the Netherlands (***K. Le Blansch** , Bureau KLB, The Netherlands*)

11:05 - 11:20 Q&As

Session 2: Measurement at the nanoscale

Chair: ***M. Dobre**, FPS Economy, SMEs, Self-Employed and Energy*

11:20 - 11:40 Tools for measurement quality at nanoscale: traceability, uncertainty, interlaboratory comparisons (***J. Pétry**, FPS Economy, SMEs, Self-Employed and Energy*)

11:40 - 12:00 Exposure to nanomaterials defining the influence of nanoparticles aggregation/agglomeration on toxicity (***P. Hoet**, KU Leuven*)

12:00 - 13:20 **Walking lunch and poster session opened by *P. Tousseyn*,** *General Director, DG Humanisation of Labour and DG Surveillance of well-being at work, FPS Employment, Labour and Social Dialogue*

13:20 - 13:30 **Afternoon's session opening speech by *G. De Poorter*,** *General Director, DG Quality & Safety, FPS Economy, SMEs, Self-Employed and Energy*

Session 3A: Nano Research in Belgium

Chair: ***C. Dantinne**, FPS Health, Food Chain Safety and Environment*

13:30 - 13:50 Nanomaterials in products: inventory, characterization and estimation of exposure via air (***H. Reynders,** Flemish Government, Department of Environment, Nature and Energy*)

13:50 - 14:10 NANO Global Risk Assessment (***E. Bouhoulle**, ISSEP*)

14:10 - 14:30 Nanomaterials in food and the microbiome (***D. Lison**, Louvain Centre for Toxicology and Applied Pharmacology*)

14:30 - 14:50 EU research policy for the safety of nanotechologies (***G. Katalagarianakis***, *EU Commission, DG Research and Innovation*)

14:50 - 15:10 Activities of the European Commission's Joint Research Centre on detection and quantification of nanomaterials in consumer products (***E. Anklam**, JRC*)

15:10 - 15:30 Coffee break

Session 3B: Nano Research in Belgium (Research & Development)

Chair: ***M. Dobre**, FPS Economy, SMEs, Self-Employed and Energy*

15:30 - 15:45 Potential toxicity of nanomaterials: case study (***J. Laloy**, University of Namur – Namur Nanosafety Center*)

15:45 - 16:00 (Epi)-genetic alterations induced by Carbon Nanotubes in vitro (***D. Oner**, KU Leuven*)

16:00 - 16:15 DNA methylation alterations in mice after single intratracheal instillation of nanomaterials (***L. Godderis**, KU Leuven*)

16:15 - 16:30 Safety characterization of gold nanoparticles in complex mixtures (***S. Deville**, VITO*)

16:30 - 16:45 Characterization of manufactured nanomaterials at different levels (***J. Mast**, CODA-CERVA*)

16:45 - 17:00 Certified Reference Materials for the Size Analysis of Nanoparticles (***R. Koeber**, JRC*)

17:00 - 17:15 Challenges in using engineered nanomaterial in semiconductor research and development (***D. Prodanov**, IMEC*)

17:15 - 17:30 Nanotechnologies and Nanomaterials – Current standardization status, challenges and opportunities (***J.P. Erauw**, Belgian Ceramic Research Centre*)

17:30 - 17:45 Closing of the meeting by ***C. Dantinne** (FPS Health, Food Chain Safety and Environment),* ***M. Dobre** (FPS Economy, SMEs, Self-Employed and Energy)* and ***L. Wouters** (FPS Employment, Labour and Social Dialogue)*

Oral presentations

The Belgian national registry of nanomaterials

Carine Gorrebeeck

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The presentation will explain why the decision was made to establish a BE nanoregistry, will explain the purposes of the registry and will lay out the principles of the registry. Furthermore, the practical modalities will be briefly introduced.

Workers' safety in the context of the Belgian nano-registry

Linda Wouters

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Workers are the first in line to be exposed during the production and use of nanomaterials.

Exposures in the working environment are more intense and more direct than the exposure of consumers who generally only come in contact with finished products.

In the workplace we see that employers-professional users of nanomaterials often lack the information they need to properly perform their risk assessment. The SDS, the most important tool for communicating the hazards of substances throughout the supply chain, frequently does not even mention the nanoform of the material. The result is that nanomaterials are often not taken into account during the risk assessment and that no nano-specific prevention measures are taken. Furthermore, nanomaterial-exposure measurements in workplaces are scarce.

The BNR will facilitate the communication of nano-relevant information throughout the supply chain, allowing the employer-professional user of nanomaterials to comply better with health & safety obligations, resulting in a better protection of workers.

The authorities will get a better founded overview on the types and quantities of nanomaterials used, the applications of various nanomaterials and the evolution of these applications, and the evolution towards the use of more complex nanomaterials. This overview is needed to set deliberate priorities for performing their duties regarding the preparation, promotion and implementation of policies on wellbeing at work.

Lessons learned from the French nano-registry

Vincent. Coissard,

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In 2007, the "Grenelle de l'environnement" anticipated the risks linked to the production and the use of manufactured nanomaterials. A few year later, the stakeholders reached a consensus on a national register with four objectives: increase knowledge of the substances at nanoscale placed on the market and on exposure scenarios, increase traceability, gather information for risk assessment and improve information to workers and to the public. This is the root of the French nano-registry which came into effect on January 1st, 2013.

Pilot Exposure Registration Working with Nanomaterials in the Netherlands

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From May 2014 to November 2015 a pilot study was carried out by the social partners in the Netherlands (Employers' organisation VNO-NCW and Trade Union FNV, with the support of IVAM UvA and Bureau KLB). The pilot study aimed to reach a practicable answer to the questions in what way a meaningful registration system of workers' exposure to nanomaterials at work can be developed, as well as what this registration should look like.

It was found that such a registration system can indeed be developed, provided that it is based on the inclusion of different types of nanomaterials (including process generated nanomaterials (PGNPs)), is triggered by Nano Reference Values, follows the Occupational Hygiene Strategy (STOP), and is integrated with existing risk management systems.

It was also found, however, that the 'meaningfulness' of such a registration system is limited. It is to be expected that industry will not comply. It will be far more effective to raise awareness and promote control measures. The effectiveness of these measures will pre-empt a registration requirement.

One of the recommendations of this study is therefore, to start an information and sensibilisation campaign on the potential risks of nanomaterials in general and PGNPs in particular, and to urge for effective control measures.

Tools for measurement quality at nanoscale: traceability, uncertainty, interlaboratory comparisons

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In the framework of the national regulation on nanoparticles, the FPS Economy, Service of metrology – National Standards started in July 2015 a pilot study on the comparability of nanoparticles size measurement at the national level. As the new regulation requires the registration of nanoparticles dimensions measured by a traceable method, with uncertainty budget associated, this comparison is also the opportunity for researchers and companies to improve comparability of their measurements through understanding of uncertainty contributions in specific measurement methods. The goal is to achieve comparability with different measurement techniques based on different physical principles. The study involves for the moment 3 different microscopy techniques (SEM, TEM, AFM) and 2 centrifugal separation (DCS) but is opened to further collaborations. First results are expected to be published in 2016

Exposure to nanomaterials defining the influence of nanoparticles aggregation/agglomeration on toxicity

Peter Hoet

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When reading the literature it is obvious that most experimental studies with NMs have been carried out with aggregates/agglomerates of NPs, even when attempts have been made to disperse the material as much as possible in the vehicles used, e.g. using different surfactants or proteins.

This has considerable repercussions on the biokinetics of the material. Several questions can be raised: what is the size distribution of the aggregates/agglomerates; what is the portion of the particles present as a monodisperse material; does a size threshold exist allowing translocation through the body or can only monodisperse particles translocate between cells/organs?

Aggregation/agglomeration influences the settling of e.g. inhaled materials in the different lung regions. Moreover, local effects are, in part, governed by the size since large aggregates cannot easily be removed (e.g. carbon nanotubes) from the initial site of deposition allowing prolonged exposure, possibly leading to chronic effects.

At the cellular level, active uptake of large aggregates – which may be larger than the cell volume – is not possible, thus reducing bioavailability for most cell types, but initiating "frustrated phagocytosis" by specific cell types.

In addition to the issues of distribution and the bioavailability of the material, intrinsic toxicity can be affected by aggregation and aggregation size too. For many endpoints it is not yet clear whether the aggregation/agglomeration plays a major role – comparing equal levels of internalized/available material – in the toxicity of the material.

In the case of amorphous nanosilica in hemolysis, this has been worked out in some detail. As mentioned earlier, the hemolytic activity of silica NPs increases with increasing size. Notwithstanding this observation, aggregates of "small" silica NPs are less potent than monodisperse materials, but the toxicity increased when the aggregates were denser and, hence, were displaying a smaller total surface area compared to the loose aggregates.

These observations demonstrate the complexity concerning surface area, size and aggregations

Nanomaterials in products: inventarisation, characterization and exposure assessment through the air

Hans Reynders[1], *Evelien Frijns*[2] , *Martine Van Poppel*[2]

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Nanomaterials are increasingly being used in the development of new consumer products worldwide. Yet, the possible risk of exposure through using these products is still largely unknown. Insight in this matter is important to (in)form policy makers about health risks of nanomaterials. For these reasons the Flemish service environment & health started a study entitled "Nanomaterials in consumer products: inventarisation, characterization and exposure assessment through the air". The goal of the study is the assessment of the possible exposure to nanomaterials in products. In a first step 8 products with a nanoclaim that are available on the market and are regularly used by consumers, will be tested on the presence of nanoparticles. For products containing nanomaterials, the exposure during use of the product will be determined by simulating representative exposure conditions. Based on these results a work plan for toxicological screening of the selected products will be made. The study also aims at formulating specific policy advises concerning potential exposure through the use of consumer products containing nanomaterials and more general conclusions on risks of nanomaterials. Based on these case-studies, tools for future exposure assessment of consumer products will be developed.

The study is performed by VITO and financed by the department of Nature, Environment and Energy of the Flemish Government.

NANO Global Risk Assessment

SINABA Tiécoura, HEMART Matthieu, ***BOUHOULLE Elodie***

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The NANO Global Risk Assessment (NANOGRA) project aims at globally assessing the risks of exposure to NMs by addressing explosion, ecotoxicological and toxicological risks. The project started in December 2014, and is funded on ISSeP's own research budget.

Explosion and fire characteristics assays will focus on the generation of reliable and comparable experimental data. In addition, they will give us some indications to which extend the laws found for powders at the micro scale remain valid at the nanoscale.

For ecotoxicological risk assessment, the environmental compartment studied will be the sediment, as it is known to be a sink for NPs reaching the aquatic medium. Standardized bioassays on Chironomus riparius and Heterociprus incongruens will be adapted to nanoscale substances. Realistic concentrations in the environment are also intended to be studied in a second phase.

The choice of nanosubstances to be studied has been made mostly for being relevant for explosion and/or ecotoxicological assessment with sediments, for their relevant use in the Walloon region and for the available knowledge of these substances in order to validate our tests development.

Control banding approach will be applied to the scenarii of Nanogra research activities.

Nanomaterials in food and the microbiome

Hélène Lecloux, Sybille van den Brule, Pieter-Jan De Temmerman, Clément Levard, Romain Soulas, Chrisitian Bailly, ***Dominique Lison***

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The gut microbiome is now considered as an entire metabolic organ fitted with many physiological functions. Many diseases, including metabolic syndrome, asthma, or allergies are associated with a disruption of the fragile balance of this ecosystem. Nanosilver (nAg) is largely used for its antimicrobial activities mainly for food packaging and diverse appliances. We examined experimentally the possible impact of nAg in food on the gut microbiome at doses relevant for human dietary exposure. Mice were orally exposed to 0, 46, 460 or 4600 ppb of nAg mixed in commercial lab pellets for 28 days. No evidence of overt deleterious effects was observed: no mortality, body weight and serum C-reactive protein (CRP) levels were not significantly affected by the treatment. No histological alteration of the gut tissues was evidenced. We found, however, a significant inversion of the Firmicutes/ Bacteroidetes ratio, as well as alpha and beta diversity alterations induced by nAg. The activity of nAg on the microbiome was reduced with time due to surface sulfidation of the nAg particles in food. These data indicate that silver nanoparticles in food can affect the gut microbiota at doses relevant for human dietary exposure.

EU research policy for the safety of nanotechologies

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Nanotechnology is referred to as the new "general purpose technology" of the 21st century, a springboard for long-term productivity increases in several sectors, for economic growth and as a means of addressing grand societal challenges. As it is normal with every new technology, concerns about its safety are raised.

It is imperative to ensure safe development and application of nanotechnologies, through a solid understanding of their potential impact on health or on the environment. Benefits from their deployment are possible only if the possibly related risks are under control.

Mindful about these concerns, the European Commission has actively promoted and supported research and development as well as innovation in this area. Through its Framework Programmes 6 and 7, and now Horizon2020, it funds a number of projects in this area as integral part of the much larger investment in nanotechnology and its applications.

Scientific research should provide the generic new knowledge to be used as scientific basis for the next steps. Regulatory research builds upon this basis for consistent development of regulations in a number of application areas and opens the way for the development of the technology and the skills necessary for implementation. The NANOREG project combines EU and national resources to implement a voluminous programme of testing of nanomaterials and of investigating exposure, aiming at providing authorities and industry with the necessary tools for the safe use of the new technologies. The NANOREG II project goes one step further for reducing risk through Safe-By-Design methods.

Research on the safety issues associated with the application of the new technologies will continue in order to achieve thorough understanding of possible risks and the capacity to manage those efficiently.

http://www.nanosafetycluster.eu

http://ec.europa.eu/research/industrial_technologies/index_en.cfm

Activities of the European Commission's Joint Research Centre on Detection and Quantification of Nanomaterials in Consumer Products

Elke Anklam

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Nanotechnology has been identified as one of the key enabling technologies for the EU. Many innovative products in development or already on the market derive from or contain nanomaterials. The introduction of such new products creates also new challenges for the legislator, particularly if the expected benefits raise concerns about health and environmental risks. There is considerable lack of knowledge about the behaviour and fate of nanomaterials. Furthermore, consumers need to be informed about the presence of nanomaterials in products on the market. Therefore, in the EU, legally binding nanomaterial definitions were adopted as part of sector-specific legislation (e.g. food and cosmetics).

The implementation of EU legislation in this area requires reliable methods for the analysis of nanomaterials according to the Commission Recommendation COM (2011) 696 which defines the term nanomaterial for legislative and policy purposes in the European Union. This nanomaterial definition creates significant challenges for implementation by the industry and control authorities. It is of utmost importance to understand whether a material needs to be labelled as nanomaterial or not. This requires reliable testing and measurement methods to determine whether a given material falls into the category 'nanomaterial'. As the results obtained in the laboratories need to be trustable, reproducible and of high quality, laboratories are requested to follow internationally harmonised and recognised standard methods for analysis which need to comply with quality criteria, e.g. accreditation according to ISO 17025.

This presentation will highlight the challenges for analysts and the need for appropriate quality assurance tools.

Potential toxicity of nanomaterials : case study

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Nanosciences and nanotechnologies are in constant evolution. Development of new therapeutic and diagnostic agents using nanotechnologies for reach their pharmaceutical target require the knowledge of biocompatibility of nanoparticles but also physico-chemical characterisation. The Namur Nanosafety Center develop methodology and contribute, with European project to the improvement of testing to be adapted to nanomaterials, in characterisation, in vitro as in vivo toxicity assessment. The case of silicon carbide (SiC) nanoparticles will be presented.

(Epi)-genetic alterations induced by Carbon Nanotubes in vitro

Deniz Öner[1], *Matthieu Moisse*[2], *Manosij Gosh*[1], *Radu Cornelieu*[1], *Lode Godderis*[1], *Diether Lambrechts*[2], *Peter Hoet*[1]

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Production and use of carbon nanotubes (CNTs) augmented the last decade, increasing the risk of exposure.

We investigated cyto- and genotoxicity and epigenetic effects induced by single-walled-CNTs (SWCNTs) and multi-walled-CNTs (MWCNTs). Two doses (25-100 µg/ml) were evaluated by means of the FpG Comet assay and the micronucleus assay with and without incubation of CytochalasinB in 16HBE (bronchial epithelial) and THP1 (monocytic) cell lines, exposed for 24h. We further, analyzed the DNA-methylation.

CNTs induced significant tail DNA and tail moment in both cells types after 24h exposure compared to controls (up to 10 fold). Moreover, oxidative DNA damage was observed. Frequency of micronucleus was increased when 16HBE cells were exposed to SWCNTs and MWCNTs (1.5 fold). No significant increase in micronucleated cells in THP1 cells were observed after 24h. CBPI (CytochalasinB block proliferation index) was not significantly altered with CytB incubation in tested conditions. However, cell cycle phases were significantly disturbed in 16HBE cells. Although, global DNA-methylation was not significantly altered after 24h exposure, gene (and cell) specific alterations were observed.

Conclusion: we observed CNTs induced DNA damage in both cell lines, clastogenic effects were only observed in 16HBE cells. Changes in DNA-methylation are complex and show gene specific alterations.

Sponsor: Stichting tegen Kanker (2012-218)

DNA methylation alterations in mice after single intratracheal instillation of nanomaterials

Ali M Tabish[1], Katrien Poels[1], Hyang-Min Byun[3], Katrien luyts[1], Andrea A Baccarelli[3], Johan Martens[4], Stef Kerkhofs [4], Sven Seys[5], Peter Hoet[1], **Lode Godderis[1, 2]***

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Epigenetic modifications play an important role in the development of chronic diseases like cancer. We investigated the effects of nanomaterial (NM) exposure on DNA methylation in BALB/c mice. We administered intratracheally gold NPs (AuNPs) of 5nm, 60nm and 250nm diameter; single-walled (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) at 0.25 mg/kg and 2.5 mg/kg for 48 hrs. Immune assays revealed AuNPs induced macrophage-dominant, while CNTs induced macrophage and neutrophil-dominant immune response. No effects were observed on oxidative stress, DNA damage, global DNA methylation and hydroxymethylation. In mice lung tissue, AuNPs 60nm exposure induced promoter CpGs hypermethylation in Atm, Cdk and Gsr genes; and promoter CpGs hypomethylation in Gpx gene. Changes in promoter methylation of Gsr and Trp53 were also observed between low and high dose of AuNPs 60 nm and AuNPs 250 nm respectively. AuNPs size effects on promoter methylation was observed for Trp53 gene; and CNTs administration affected the promoter methylation of Atm gene. In mice blood DNA, we observed promoter hypermethylation in Pparg gene after administration of AuNPs 60 nm high dose compared to low dose. In conclusion, the current study revealed epigenetic machinery is susceptible to NM administration.

Safety characterization of gold nanoparticles in complex mixtures

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The medical application of gold nanoparticles (GNPs) is promising due to their high biocompatibility, but little is known about their behavior in mixtures with other chemical compounds against a complex biological background. We aimed to study the safety profile of GNPs when combined with nickel, a widely distributed heavy metal that forms alloys with gold, and has allergenic and carcinogenic potential.

We focused on allergic sensitization by evaluating in vitro activation of dendritic cells. The physico-chemical interactions between 50-nm GNPs and nickel(II)sulphate in biological matrix were investigated using nanoparticle tracking analysis, centrifugal particle sedimentation, UV-Visible spectroscopy, ICP-MS, ζ-potential determination, and proteomics.

While both GNPs and nickel(II) induced a sensitization response, the cell activation pattern of their combination was similar to this of nickel(II), suggesting a competitive interaction. Characterization data indicated that nickel(II) ions did not adsorb onto the GNP surface, but caused a clear shift in the GNP-adhered protein corona composition in biological medium. ICP-MS analyses demonstrated a significant decrease in GNP uptake by the cells in the presence of nickel(II).

This study highlights the necessity of assessing nanomaterials' health risks in a complex environment reflecting the real world, and the need to complement such studies with in-depth physico-chemical characterization.

Characterization of manufactured nanomaterials at different levels

Jan Mast

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The properties of a nanomaterial, as those of any material, strongly depend on the chemical composition, the crystal structure and the phase. Due to the quantum effects and the increased volume-specific surface area (VSSA) at the nano-scale, physico-chemical properties can be different from the properties of the material in bulk form. Measurands estimating the minimal external dimension of the primary particles correlate well with the VSSA and are suitable to define a nanomaterial. The size, shape and surface properties of primary particles, and also of aggregates and agglomerates of nanoparticles determine their influence on biological systems. They are essential to evaluate the efficient application of nanomaterials and to interpret their nanocytotoxicity and risk analyses. Identification of a specific nanomaterial in a complex matrix requires the simultaneous measurement of its physical (size, shape) and chemical properties (composition).

These concepts are illustrated for selected examples based on characterization data acquired at different levels in several (inter)national research projects using validated characterization methods including transmission electron microscopy, single particle – inductively coupled plasma – mass spectroscopy and particle tracking analysis.

Certified Reference Materials for the Size Analysis of Nanoparticles

Robert Koeber, *Gert Roebben, Vikram Kestens, Thomas Linsinger, Hendrik Emons*

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The characterisation of the properties of nanomaterials requires advanced measurement methods. Certified reference materials (CRMs) are the much needed tools to monitor the performance of these methods and to help laboratories develop confidence in their measurement results. To support the laboratories measuring and testing nanoparticles, the JRC's Institute for Reference Materials and Measurements (IRMM) in Geel (BE) develops nanoparticle reference materials with certified average particle sizes based on relevant industrially produced particles. At first, CRMs of spherical nanoparticles with monomodal size distributions were produced and certified. In 2014, the first mixture of two colloidal silica materials was released that can challenge methods whether they can cope with multimodal particle size distributions. Future developments will be focused on irregular shaped, polydisperse particles with broad size distributions, both as pure particles or embedded in a matrix. Some of these potential future materials could be sourced from the European research project NANODEFINE that supports the implementation of the EC Definition of a Nanomaterial. In order to assure the high technical and quality level of its products, JRC-IRMM is constantly looking for collaborators for projects on stability monitoring and certification of new reference materials.

Challenges in using engineered nanomaterial in semiconductor research and development

D. Prodanov[1], *J. Coenen*[2], *A. Pardon*[1]

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Measurements of engineered nanoparticles (ENP) emissions are challenging because of the lack of exposure standards and monitoring instruments. To manage unforeseen risks, related to possible exposure to nanomaterials the safety team in Imec has deployed a tiered approach, known as risk and control banding. This approach is based entirely on the precautionary principle. Hazards of nanomaterials are classified in three groups, based on the properties of the parent material and the physicochemical properties of the nanophase. Risks related to the operations are also classified in three groups based on the likelihood of the release. This classification is co-related to monitoring of particle content in the air. Proposed approach has been also considered by some semiconductor companies. ENPs are increasingly being used in nanoelectronics industry as abrasives in the process of Chemical Mechanical Planarization (CMP), in addition there are ongoing efforts to use them as interconnect materials. Accidental release of such materials is possible at different points along the trajectory in a Fab facility. In order to investigate this we have performed pilot studies investigating different properties of the release in the cleanrooms of Imec. The presentation will discuss some of the issues related to dealing with uncertainty in R&D settings.

Nanotechnologies and Nanomaterials – Current standardization status, challenges and opportunities

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Nanotechnologies are key enabling technologies that may have an impact on numerous industrial sectors as e.g. chemical engineering, energy production and storage, medicine and healthcare products, ICT, materials and consumer products …

Despite the real opportunities for growth and development in these sectors and the resulting attractiveness of those emerging technologies and products, concerns still exist about the potential hazardous effects of engineered nanomaterials and products containing them.

In this context, standardization is seen as a necessary step for a safe, integrated and responsible approach as recommended by the EC, hence the publication in 2010 of the "Standardisation Mandate to CEN, CENELEC and ETSI for standardisation activities regarding nanotechnologies and nanomaterials".

The presentation aims at providing an insight in the current standardization activities in the fields of nanotechnologies and nanomaterials. Main current standardization projects at the European and international level will be briefly described, a focus being given at the by the EC mandated items.

The role and working of the Belgian mirror committee I229 will be detailed.

Poster presentations

ISSeP: Tools available in the Nano field

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ISSeP is the reference lab for Air, soil and water quality in Wallonia. The institute is running different networks in Wallonia which include more than 50 stations for air, 300 points of measurement for water … One station and two trailers are equipped with SMPS and allow measurement of ultrafine particles in ambient air in the range 10nm to 850 nm. The institute also routinely runs a large battery of ecotoxicology tests which could be used to evaluate the impact of nanos released in the environment. Several tools are available to observe the structure of nanomaterials using different techniques of microscopy. The risk department can also realize different risk evaluation studies regarding the impact of nanos on human health and environment. All these tools have been already used in different European projects or to answer specific questions arising from private customers. ISSeP is a strong partner in metrology and remains available for new collaborations in the nanofield.

Silver nanoparticles interference with classical cytotoxicity assays

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Because of their small size and high reactivity, nanoparticles can be more toxic than their bulk materials. Owing to their antimicrobial properties, silver nanoparticles (AgNPs) are the most used in consumer products, e.g. in medicines, cosmetics or food. However, some gaps remain about their potential toxicity. In vitro models could help to address these questions, being more rapid and rentable than in vivo assays. Moreover, defined toxicity endpoints can be studied, such as mitochondrial or lysosomal disturbances. Nevertheless, because of their high reactivity, nanoparticles could interfere with assays and lead to a bias in the toxicity assessment. Nanoparticles interact either directly with assay components such as reagent, enzyme or surfactant. Alternatively, they can trigger a cellular response, which in turn will interfere with the assay. In our approach, we have shown that classical cytotoxicity assays such as LDH, ATP luminescent measurement and MTT can not be used to estimate the toxicity of AgNPs while no interference has been proven between AgNPs and neutral red or resazurin assay.

Monitoring the intracellular dynamics of polystyrene nanoparticles in lung epithelial cells by image (cross-) correlation spectroscopy and single particle tracking

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Interactions of nanoparticles (NPs) with living cells and resulting biological responses are largely dependent on NP uptake processes, intracellular transport and their complex behaviours. In order to demonstrate the applicability of image (cross-) correlation spectroscopy based techniques for monitoring the intracellular dynamics of NPs, 100 nm fluorescently stained carboxylated polystyrene (PS) NPs were used to expose in vitro cultured human lung epithelial A549 cells, thus mimicking NP exposure in the lungs. Transport direction, transport velocity and diffusion of PS NPs were determined to acquire more insights in the intracellular transport following NP uptake. To complement these ensemble average techniques, PS NP motions were also analysed by single particle tracking. Hereby, distinct clusters are registered and tracked frame by frame allowing access to individual PS NP dynamics. Potential dynamic interactions of NPs with the nucleus, mitochondria, early endosomes, late endosomes and lysosomes were also explored. PS NPs directed motions were shown to be highly dependent on the microtubule-assisted transport and were strongly associated with the endolysosomal compartment. Image (cross-) correlation analyses were shown to be a powerful tool for determining the kinetic behaviour of NPs inside the cell.

[1] Penjweini, R.; Smisdom, N.; Deville, S.; Ameloot, M., Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, **1843 (5)** (2014), 855-865.

[2] Deville, S.; Penjweini, R.; Smisdom, N.; Notelaers, K.; Nelissen, I.; Hooyberghs, J.; Ameloot, M., Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1853 (10) (2015), 2411-2419.

FP7 QualityNano Transnational Access case studies at VITO's exposure assessment facility

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The QualityNano project (2011-2015) integrated 28 top European analytical and experimental facilities in nanotechnology, medicine and natural sciences with the goal of developing and implementing best practice and quality in all aspects of nanosafety assessment (http://www.qualitynano.eu/). The Transnational Access (TA) component was dedicated to providing Users from the European nanosafety community access to nanomaterials (NMs) processing, characterisation and exposure assessment facilities.

A selection of two TA studies performed at VITO will be presented in this poster. The first case study is related to the cytotoxicity assessment of a novel CdTe quantum dots (QDs)-based fluorescent ink. The main target was to characterize airborne emissions of the QDs-based ink during printing focussing on potentially released NMs, and to determine their toxicological effect through in vitro studies using airway cells cultured at the air-liquid interface and in conventional submerged conditions.

In the second case study nanoparticle release during drilling of graphene/epoxy/fibre reinforced nanocomposites was studied. This involved the use of real-time measurement and characterization instrumentation at VITO, including CPC, SMPS and DMS50.

Evaluation and measurement of exposure to inhalated nanoparticles in the workplace

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This poster presentation will give an overview of VITO's current running projects related to the evaluation and measurement of exposure to inhalated nanoparticles in the workplace.

To assess the potential for inhalation exposure to nanoparticles and nanostructured materials VITO applies a **tiered approach** which is demonstrated in two industrial case studies within the LIFE+ NanoRisk project. As an alternative to the first tier, **project control and/or risk banding tools** are applied in the FP7 Dibbiopack , providing a ranking of activities with respect to potential for exposure. The necessity of exposure monitoring as a complementary tool to risk banding is studied for the semiconductor industry in the H2020 NanoStreem project.

Within the SIM Flanders Met@Link project (industrial printing) and the H2020 NanoTun3D project (additive manufacturing) VITO carries out the **safety-by design concept** applying the precautionary principle in the early stage in industrial R&D processes. These include risk assessment studies along the life cycle, exposure measurement and characterization (on-site or under simulated conditions in test chambers), human and/or environmental safety testing, and advise on risk management strategies.

Tailor made strategies for source identification and exposure monitoring and mitigation in occupational settings are provided for industry, SME's and government. One industrial case-study will also be presented.

Development of best practice in nanosafety assessment via the FP7 QualityNano Research Infrastructure

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Quality and relevance of nanosafety studies constitute major challenges to ensure their key role as a supporting tool in sustainable innovation and subsequent competitive economic advantage. In the FP7 QualityNano Research Infrastructure training modules have been organized and best practices in nanosafety research disseminated as part of its networking activities. To this end standard operating procedures (SOPs) for in vitro assessment of established biological endpoints have been developed and validated in a multi-step process. By means of round robins in a small group of expert laboratories SOPs have been optimized and verified, to disseminate them to the nanosafety research community. The SOPs have further been used for interlaboratory comparison (ILC) studies among >10 laboratories to evaluate proficiency of the participating laboratories, and to test candidate positive and negative control nanomaterials as assay controls and determine their response ranges. Based on the outcome of ILC studies training needs for research laboratories have been identified and SOPs refined further. The results of these pre-normative activities will finally be proposed for standardization. Such process has been adopted for determination of nanomaterial-induced cytotoxicity using the MTS assay and will be presented here. Similar activities related to other biological assays will be highlighted.

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