

***ESF Human Nanotoxicology Meeting:
London 22-23rd September 2011***

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1) Meeting Introduction and Summary

Nanotoxicology is the developing study of naturally occurring and engineered nanomaterials interacting with biological systems. Such interactions may be positive (e.g. in drug delivery) or negative (e.g. causing inflammation). Human nanotoxicology examines the mechanics of interference of nanomaterials with cellular nanostructures or whole organisms, and includes the use of human and animal models. Nanomaterials have unique properties that make them promising for many medical applications, including antimicrobial dressings, *in vivo* imaging, tissue regeneration, plus drug and gene delivery, but it is essential that applications are developed in parallel with investigations of their safety.

Nanoparticles (NPs), engineered nanoparticles (ENPs) and particulate matter (PM) first come into contact with the respiratory, digestive and cardiovascular systems during human exposures. NP/ENP/PM surfaces first interact with molecules within these systems and this may determine subsequent biological fate. This meeting discussed these surface interaction concepts and the implications for human health, through European Science Foundation supported international dialogue.

Recently it has emerged that many of the biological processes involving nanomaterials are common to diverse disciplines. For example, nanomaterials studies have demonstrated that proteins adsorb to the material or particle surface immediately upon contact with the lung or blood, in particular to reduce the surface energy of the exposed material surface. Subsequently the nanomaterial surface itself is no longer exposed but rather a layer of adsorbed biomolecules is presented at the bio-nano interface. In terms of bioactivity, the important parameter is thus the conformation and structure of the adsorbed protein layer, and in particular, the very outer protein layer, as this is what cells interact with. The removal of certain molecules from biological fluids via these means may also have significant downstream consequences for the organism, via such factors as nutrient or protein depletion, or alterations in enzymatic activity as a consequence of being bound to nanoparticles.

The key academicians targeted to attend this meeting were from a diffuse, cross-disciplinary group of scientists and medics working specifically in some aspect of human nanotoxicology. This meeting was the first ESF meeting exclusively targeted at human nanotoxicology, where different target organs were discussed during different sessions using evidence from human studies. Academics from around Europe with experience in this area were invited to attend the meeting, and several Professor-level academics, and younger scientists-in-training gave presentations of their work, and the work of their group. The result was a stimulating discussion between academic disciplines, which highlighted the similarities across seemingly diverse research spheres.

1) Meeting Scientific Content and Discussion

Human Exposures to NPs and ENPs

The host and Co-organiser Dr Michaela Kendall (European Centre for Environment and Human Health [ECEHH], University of Exeter, Exeter) presented an introduction to the meeting and outlined the meeting aims. She then presented the first talk on “*Human NP and ENP Exposures: Documented Effects*” which integrated another talk by Dr Jess Tyrrell, (ECEHH, University of Exeter, Exeter) “*Medical applications of ENPs and effects on human platelets*”. The key message was that NP exposures had great parallels with environmental exposures to airborne particulate matter from combustion sources, especially in cities in developed countries. A great deal can be learned from this vast body of work, especially when discussing exposures to the lung. Another message was that nanomaterials are not new and that the body has developed defensive mechanisms to these materials at background environmental doses. It is when these “natural” nanoparticle doses are exceeded during acute and chronic exposures that effects can be observed. The second talk reviewed *in utero* exposures to nanomaterials by Dr Margaret Saunders (BIRCH, Biophysics Research Unit, St Michael’s Hospital, Bristol). The presentation “*Models of In Utero NP Exposures: Evidence and prospects*” reviewed recent works, results and papers, and gave an update of on-going work by Dr Saunders. Nanoparticle exposure during pregnancy is an area that has received little attention but is of great importance due to the vulnerability of the developing foetus. Her group has experience in this area and is developing a range of models in order to determine potential foetal exposure levels and assess nanotoxicity. The final talk in the session was by Prof Antonietta Gatti (Laboratory of Biomaterials, University of Modena, Italy), entitled “*Nanopathology: The human nanotoxicology*”. Professor Gatti has published widely on her findings of nanomaterials in tissue retrieved for pathologic study. Nanoparticles have the property to cross the physiological barriers as a result of their tiny size, and because of this property can be dispersed in all the parts of the body and accumulate to induce foreign body reactions. The lecture presented images of engineered and not engineered nanoparticles that were present in pathological samples coming from individuals exposed to nanoparticles under diverse conditions, including during warfare, discussing the possibility of clearance of these nanoparticles.

ENPs in the Human Gut

Prof Jonathan Powell (MRC Human Nutrition Research [HNR], Cambridge, UK) presented “*Nanoparticles and Nanominerals in the Gut*”. Jonathan is Head of Biomineral Research (formerly Micronutrient Status Research), Visiting Professor in the School of Medicine at King's College London and is a Fellow of Hughes Hall, Cambridge. The Biomineral Research section concentrates on the role of minerals in health and disease. It has four major research programmes; Dietary Silicon and Health, Dietary Inorganic Microparticles in Gut Health, Trace Element Status and Analysis, and Iron and Novel Synthetic Minerals. Jonathan's presentation focused on (i) the absorption of iron and diagnosis and treatment of iron deficiency, with a particular focus on novel mineral-based iron supplements, and (ii) the role of minerals, especially nano- and micro-sized mineral particles, in inflammatory and anti-inflammatory processes of the gastrointestinal tract. He discussed issues of mineral metabolism and analysis, especially silicon and its potential role in biology. Dr. Andreani Odysseos (University of Cyprus, Cyprus) continued this human gut

theme by presenting on “*Co-axial nanofibers with tunable release properties as drug delivery and tissue engineering platforms*”. She introduced work on reproducible and sustainable drug/growth factor delivery nanosystems based on biodegradable polymer nanofibrous scaffolds fabricated via co-axial electrospinning (i) for *in situ* applications and (ii) as platforms for controllable release in intestinal tissue engineering. α -Tocopherol, a minimally toxic, lipid-soluble natural agent with well-defined signalling, growth and differentiation pathways is used as a prototype agent combining both therapeutic and growth/differentiating properties and demonstrating special predilection for intestinal epithelial. Understanding the desirable interactions such as these can also provide key insights for preventing uptake by similar mechanisms of nanoparticles not intended for uptake by biological systems.

ENPs in the Human Lung

Dr Jens Madsen (University of Southampton, Southampton) presented “*In Vitro Models of NP Exposures: Evidence and prospects*”. He described *in vitro* human cell culture models that can be used to determine human NP doses in terms of lung cell viability and function. In Southampton School of Medicine these models are combined with viral or bacterial infections models to simulate a 'real life' multi-ligand exposure model testing potential harmful or beneficial effects of NPs. This project is supported by the MRC-ITTP scheme and co-supervised by Prof Clark and Dr Kendall. Dr Andrew Thorley (Imperial College, London) described a range of ongoing studies examining the “*Mechanisms of nanoparticle toxicity in the peripheral lung*”. The research focused on the factors affecting the toxicity of NPs in the respiratory alveolar region. Using primary human alveolar macrophages and type II epithelial cells and a unique alveolar type I epithelial cell line, recent research has focused on mechanisms of uptake of NPs by the alveolar epithelium and how NPs modify the host innate immune response. Prof T Tetley picked up this theme later, in the session on Human Immunology on the second day of the workshop.

Biophysical Processes in ENP Translocation, Fate and Effects

The second day was started by a presentation from Dr Iseult Lynch (UCD, Dublin, Ireland). The presentation “*Protein-ENP Interaction as ENP Effect Mediator*” examined the role of proteins as the mediator of nanoparticle uptake, fate and behavior and predictive nanomaterial fate and behavior based on their protein coronas. Other important biomolecular corona components include lipids and polysaccharides, although less has been done to investigate these and their roles in nanoparticle uptake and localization. Understanding these interactions will provide researchers with the keys to unlocking nanomedicine and ensuring nanosafety by design. Dr Samir Nuseibah (Imperial College, London) presented further work from the Tetley group on “*In vitro toxicity effects of metal oxide ENPs*”, where they have developed *in vitro* models of the alveolar unit using primary human cells. Using this system, he studied the toxicity and bio-reactivity of metal oxide NPs and demonstrated that CuO and ZnO NPs have a narrow dose range of toxicity, likely linked to their solubility. Dr Pakatip Ruenraroengsak (Imperial College, London) presented on the “*Effects of size and surface modification of ENPs on cell toxicity*”. The research focused on the effect of size and surface modification on the ability of NPs to induce toxicity, oxidative stress and inflammation in cardiomyocytes, human alveolar epithelial cells and macrophages; his results showed that surface modification can drastically alter the reactivity and toxicity of NPs. Unfortunately the talk by Dr Eniko Kadar (Plymouth Marine Laboratory, Plymouth) on the

“Development of a versatile, “nano-sensitive”, non-invasive, reproducible and rapid nanotoxicology tool” was cancelled at short notice and could not be substituted.

The Role of ENPs in Human Immunology

Dr Rosana Simón-Vázquez (Biomedical Research Center [CINBIO], Universidad de Vigo, Spain) presented on the *“Cytotoxicity and immunogenicity of nanomaterials: Studies at the cellular and molecular level”*. Cytotoxic effects exerted by Nanomaterials (NMs) were characterized in their laboratory at cellular and molecular level, using human and mouse cell lines. Inhibition of different phagocytic routes demonstrated how NPs are internalized via several pathways, including phagocytosis and pinocytosis. This laboratory has also experience in testing the immunogenic response of polymeric-based nanovaccines. Prof Howard Clark (University of Southampton, Southampton) presented on *“Murine Models of Respiratory Innate Immunity: Evidence and prospects on NP Exposures”*. His group use *in vivo* mouse respiratory models looking into innate immunity of the lungs. These models are being used to investigate *in vivo* NP doses and the effect on clearance and biotransformation to other tissues. Combined with viral or bacterial infections models they test the potential harmful or beneficial effects that NPs could have on the immune system of the mouse. Prof Teresa Tetley (Imperial College, London) discussed *“Mechanisms of nanoparticle toxicity in the peripheral lung”*, focusing on the factors affecting the toxicity of NPs in the respiratory alveolar region. Using primary human alveolar macrophages and type II epithelial cells and a unique alveolar type I epithelial cell line, recent research focused on how NPs may modify the innate immune response.

Nano Law, Ethics and in Society

Prof Ilise L Feitshans (Geneva School of Diplomacy, Geneva, Switzerland) presented on *“FORE CASTING NANO LAW: Risk management protecting public health”*. The momentum from these revolutionary new nontechnology products can also bring a revolution for public health. Ilise questioned how the benefits of nanotechnology can be realized while minimizing risk, and projected how national and international law may respond to these developments. Dr Yasemin Erden (St Mary’s University College, London) discussed the *“Ethical, social and legal dimensions of nanomaterials”*, arguing that ethical, social and legal issues regarding NPs and technology should remain at the fore when developing nanovaccines and medicines. In a recent stakeholder session (NanoImpactNet, Lausanne, 2011) on nanovaccines, some of the key issues discussed included:

- Benefits (specific advantages, both medical and social)
- Risk and uncertainties (risk evaluation, management and perception; bioaccumulation and toxicity; standardisation)
- Patient involvement (upstream)
- Justice and inclusiveness (inequalities of access; legal and regulatory challenges).

Dr Elena Simakova (The Business School, University of Exeter, Exeter) considered *“Nanotechnology between science and society”* as an ethnographer. She studied conversations taking place at the university-industry-policy interface in the US, and how policy concepts (such as university-industry collaboration; or societal relevance of research) were interpreted by scientists in emerging fields such as nanotechnology and nanosafety. She also examined the challenges for social scientists researching these emerging fields. She presented on her most recent project about issues concerning the practices of producing and capturing the impact of scientific work in the areas such as nanoscience and synthetic biology.

3) Assessment of Results and Impact

The meeting was held at the London headquarters of the Chartered Institute of Environmental Health, which served as an ideal location. All presentations were in Powerpoint, and allocated to one session to ensure that presentations focussed on the required topic, with each session ending with an interactive discussion. The instructions given to presenters were:

- Preceded with a 3-5 slide general introduction to your area of expertise
- Delivered within time (normally 20 mins), with 5 minutes for questions

Each participant was encouraged to contribute to the final interactive session. Presentations were encouraged to be accessible to a general technical audience outside the scientists' own field and every presenter was encouraged to attend as many sessions as possible to engage with investigators from different disciplines and contribute to the final interactive session. Reimbursement of reasonable expenses were made in accordance with ESF rules and previous instructions by ECEHH at University of Exeter.

In this meeting, we brought together scientists and experts from different disciplines related to nanomaterial characterisation, use and effects in human systems (lung, cardio vascular, lymph, immune). We addressed the common challenge of understanding the factors that drive nanoparticle interactions with, and impacts on, human organs and systems. We discussed the effect of this interaction on biocompatibility and nanoparticle toxicity, and some general scientific paradigms emerged. A final session examined the implications for nano ethics, law and regulation, where experts in legal frameworks and ethics will help to develop a rational definition and framework for nanomaterials. Our goal was to define whether commonalities exist between the different routes of human exposure to nanomaterials, specifically in terms of the types of human bio-interactions and human health outcomes. This was achieved by encouraging focused dialogue between disciplines to increase broad understanding of common processes, develop a rational basis for risk assessment, and to reduce in the barriers to developing commercial applications of biomaterials and nanoparticles.

This meeting brought together leading representatives of a range of disciplines with relevance to these processes in humans. We encouraged communication between traditionally distinct research areas by designing a programme with sessions that included three or more academics from the different areas to address a session theme.

Several new research collaborations have resulted from this meeting, such as the subsequent visit of Ilise Feitshans to University College Dublin for 8 weeks (also funded via the ESF EpitopeMap programme) and the collaboration between Dr. Michaela Kendall and Ilise, which has resulted in a draft paper. Additionally, Dr. Margaret Saunders was invited to participate in the joint EU FP7 NeuroNano – ESF EpitopeMap workshop on nanoparticle interactions with biological barriers held in Dublin on 6th and 7th December 2011, where she presented further details on her work with *in vitro* and *ex vivo* foetal barriers, and synergies with *in vitro* Blood brain barrier models were discussed.

PROGRAMME OVERVIEW

Day 1		Thursday 22nd September 2011
8:30	Registration and coffee	
9:30	Welcome and Introductions	
10:00-10:30	Interactive session	Defining Nano
<i>10:30-11:00</i>	<i>Break</i>	
11:00-12:00 Human Exposures to ENPs (MK)		
11:00-11:20	<i>Medical applications of ENPs</i>	J Tyrrell (ECEHH, U of Exeter)
11:20-11:40	<i>Models of in utero NP exposures</i>	M Saunders (BIRCH, Bristol)
11:40-12:00	<i>Human nanopathology</i>	A Gatti (U of Modena)
<i>12:00-1:30</i>	<i>Lunch</i>	
1:30-2:20 ENPs in the Gut		
1:30-2:00	<i>ENPs in the Gut</i>	J Powell (U of Cambridge)
2:00-2:20	<i>Co-axial nanofibers with tunable release properties</i>	A Odysseos, (U of Cyprus)
<i>2:20-2:35</i>	<i>Break</i>	
2:35-3:15 ENPs in the Lung		
2:35-2:55	<i>Mechanisms of nanoparticle toxicity in the peripheral lung</i>	A Thorley (Imperial College)
2:55-3:15	<i>In vitro models of NP Exposures</i>	J Madsen (U of Southampton)
3:15-4:00	Interactive session	Groups rank human NP and ENP exposures
4:00-4:30		<i>Presentation of group findings</i>
4:30	<i>Close</i>	<i>Local dinner</i>
Day 2		Friday 23rd September 2011
8:30	Registration and coffee	
9:00-10:30 Biophysical processes in ENP translocation, fate and effects		
9:00-9:30	<i>Protein-ENP interaction as ENP effect mediator</i>	I Lynch (UCD Dublin)
9:30-9:50	<i>In vitro toxicity effects of metal oxide ENPs</i>	S Nuseibah (Imperial College)
9:50-10:10	<i>Effects of size and surface modification of ENPs on cell toxicity</i>	P Ruenraroengsak (Imperial)
10:10-10:30	<i>Development of a nanotoxicology</i>	E Kadar (PML)

	<i>tool</i>	
<i>10:30-11:00</i>	<i>Break</i>	
11:00-12:20	Immunology	
11:00-11:20	<i>Cytotoxicity and immunogenicity of nanomaterials</i>	R Simón-Vázquez (CINBIO, Vigo)
11:20-11:50	<i>Murine models of respiratory innate immunity</i>	H Clark (U of Southampton)
11:50-12:20	<i>Immune responses to nanoparticles in the peripheral lung</i>	T Tetley (Imperial College)
<i>12:20-1:30</i>	<i>Lunch</i>	
1:30-2:15	Interactive session	Groups rank human effects
2:15-2:45		<i>Presentation of group findings</i>
<i>2:45-3:00</i>	<i>Break</i>	
3:00-4:00	Nano Law, Ethics and in Society	
3:00-3:20	<i>Forecasting nano law</i>	I Feitshans (Geneva School of Diplomacy)
3:20-3:40	<i>Ethical, social and legal dimensions of nanomaterials</i>	Y Erden (St Mary's Uni. College)
3:40-4:00	<i>Nanotechnology in between science and society</i>	E Simakova (U of Exeter)
4:00-5:00	Future collaborations	
<i>5:00</i>	<i>Close</i>	