

## Strengthening communication amongst nanosafety researchers in the UK: Report from the first annual SnIRC day

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The Safety of Nano-materials Interdisciplinary Research Centre ( SnIRC ) held its first annual meeting on the 12th June at Napier University, Edinburgh. The day was organised by Professor Vicki Stone of Napier University together with SAFENANO, and brought together SnIRC members from the Institute of Occupational Medicine, Edinburgh University and Napier University. As well as providing an opportunity for members to gather, exchange ideas and discuss the current state of play in the field, it offered a platform for research students involved with SnIRC to present their most recent findings to the group, and to receive feedback on their current work and latest ideas from the experts in attendance.



Following a welcome by Professor Vicki Stone, Dr Rob Aitken, SnIRC's Director, opened the session with an introduction to the initiative. SnIRC aims to become the leading UK and European centre for information, advice on health, safety and environmental impacts of nanoparticles. It is hoped that by generating a scientific evidence base for management of NP risks through integration with other UK and international researchers and regulators, the growth of the UK nanotechnology industry may be facilitated in parallel to safeguarding workplace, public and environmental health. Furthermore, SnIRC actively assists Government Departments in developing sound strategies for managing public, workplace & environmental health and safety.

The first scientific session of the day focussed on recent research into the toxicology of carbon nanotubes (CNT). Professor Vicki Stone, head of Napier University's nanotoxicology initiatives and SAFENANO's Director of Toxicology gave a presentation on recent work at Napier University investigating the toxicity of short, long and entangled CNT in macrophages. After outlining the mechanisms of asbestos induced lung disease and why toxicologists are so interested in the parallels between asbestos morphology and CNT, Prof Stone provided a summary of a study examining CNT and nanofibre uptake by macrophages, which was carried out in partnership with the Manchester, Cambridge, Nottingham, Edinburgh and Bern Universities.

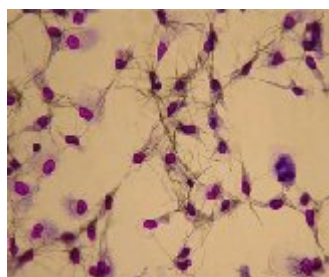
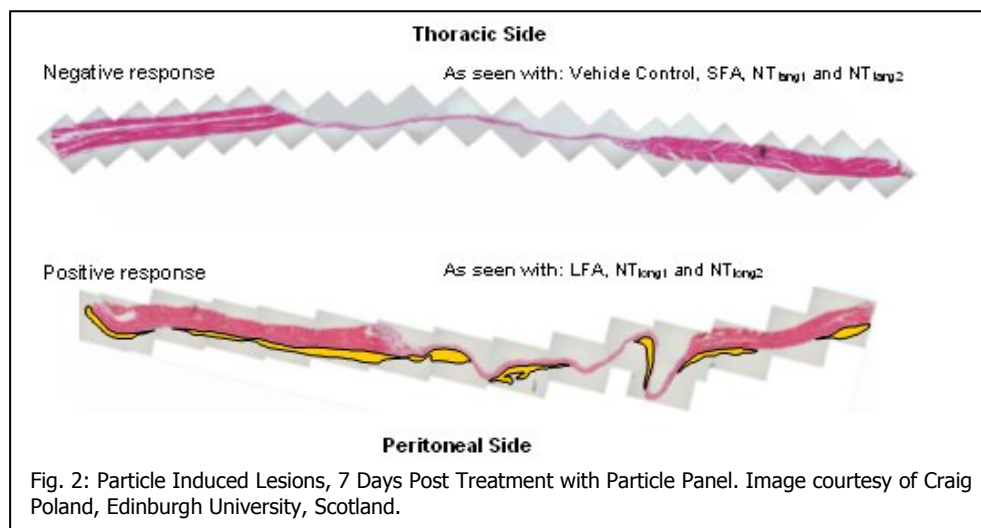


Fig. 1: NT1 CNT uptake by macrophages. Image courtesy of David Brown, Napier University, Scotland.

In the study, a series of macrophage uptake and cytotoxicity studies were undertaken using three types of CNT (long & straight, short, and tangled) and two types of nanofibres (herringbone & platelet). Results from a series of initial cytotoxicity tests, which included an LDH assay, superoxide anion production by PMA primed macrophages, inhibition of phagocytosis and induction of TNF $\alpha$  expression, allowed Prof. Stone to outline of the extent of cell damage following exposure to CNT. To date, the group's findings indicate nanotube shape to be a key determinant of macrophage response - long straight fibres are not readily ingested by

macrophages, induce a phagocytic 'burst', stimulate TNF $\alpha$  expression and are thus comparable in behaviour to long fibre amosite; whereas entangled nanotubes or shorter nanofibres are readily phagocytosed by macrophages and do not stimulate superoxide anion or TNF $\alpha$  expression. A detailed account of this work may be found in [Brown et. al., 2007, Carbon 45, 1743-1756](#).

Following this Craig Poland, author on the widely publicised Nature Nanotechnology paper '[Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study](#)' provided an overview of the research behind this paper. The work, which was led by Professor Kenneth Donaldson at the [University of Edinburgh](#), aimed to explicitly test the hypothesis that long carbon nanotubes behave like long asbestos fibres. During the study, five representative materials were tested: short amosite asbestos fibres, long amosite asbestos fibres, short and/or tangled MWCNT, long straight MWCNT, and carbon black (compact graphite-based particles). Samples of each material were injected into the abdominal space of mice, and subsequent inflammation and the formation of granulomas in the lining tissue (the mesothelium) was studied.



The group's primary finding was that exposing the mesothelial lining of the body cavity of mice to long multiwalled carbon nanotubes (MWCNT) resulted in length-dependent, pathogenic behaviour. Long MWCNT were shown to behave like long asbestos, inducing rapid inflammatory and fibrogenic effects in a model of direct mesothelial exposure. This allowed the authors to conclude that long MWCNT behave in a manner concurrent with that of the fibre toxicity paradigm. The group found that exposure to short and / or tangled nanotubes was less likely to result in disease than exposure to long, straight fibres and that the longer, straighter and more fibre-like the CNT, the more pathogenic it is likely to be. However, Craig was quick to stress that further work is required to address whether exposure to long straight carbon nanotubes will occur or, if it does, whether these fine fibres will reach the mesothelium surrounding the lungs, and go on to cause mesothelioma. In May, SAFENANO featured an overview and commentary on the findings of this study from its lead scientist, Ken Donaldson. For further information on this important study, please [click here to access Ken's article](#).

The final presentation of the CNT toxicology session came from Matthew Boyles of Napier University. Matthew presented the results to date from his ongoing study examining the ability of multiwalled Carbon Nanotubes to generate Reactive Oxygen Species (ROS) independently, both *in vivo* and *in vitro*. In his work, cell free suspension media, J774.A1



macrophage and bronchioalveolar lavage cells were treated with three different types of MWCNT (long+straight [NT1], short+aggregated [NT2] and long+tangled [NT3]), and the generation of ROS was measured using a fluorescent probe - DCFH (2',7'-dichlorofluorescein). His preliminary results indicate that although all NTs increase ROS production significantly over a 4 hour incubation period, there are definite particle type dependant differences, the greatest increase in generation coming from treatment with entangled MWCNT (NT3).

The second session focussed on the ecotoxicology of nanoparticles, commencing with a presentation from Philipp Rosenkranz of Napier University. Philipp's work concentrated on an examination of the toxicity of carbon black and silver particles as a function of mass dose and surface area in the freshwater organism *Daphnia magna*. Acute exposure tests to carbon black and silver were conducted using the endpoints of mortality and moulting. Mortality results for carbon black exposure suggest a positive relationship with surface area dose indicating that its toxicity is surface area related. In comparison, results for silver suggest that surface area dose is not the only factor responsible for the mode of silver toxicity. Moulting results obtained support the conclusions derived for mortality data.

Jon Mullinger, who is currently undertaking a PhD at Napier University then presented his work on the ecotoxicology of nanoparticles with particular reference to the importance of relevant exposures. His work aims to investigate environmentally realistic exposure scenarios for nanoparticles and test the ecotoxicology of nanoparticles using those exposure scenarios selected as suitable. Although Jon's work is currently at a relatively early stage, it has so far shown that humic substances (major constituents of soil organic matter humus that contribute to soil chemical and physical quality) are able to induce stability in TiO<sub>2</sub> nanoparticle solutions, that TiO<sub>2</sub> NP stability occurs at concentrations of humic substances that are found in the environment, and that at these concentrations they are not acutely toxic to *daphnia magna*, the representative organism chosen for investigating potential ecotoxicity.



Fig. 4: Monitoring *D. magna* health following 4 days of exposure to humic acid. Image courtesy of Jon Mullinger, Napier University, Scotland.

Vicki Stone then presented results of work carried out by Birgit Gaiser of Napier University on the ecotoxicology and toxicology of water-born nanoparticle exposure. In Birgit's study, the toxicity of silver and cerium dioxide nanoparticles compared to bulk particles was investigated in the planktonic crustacean *D. magna*, the cyprinid *C. carpio* and human liver cells. *In vivo* tests were undertaken to assess the endpoints of mortality, growth and moulting in *D. magna*, while the cytotoxicity was examined in human hepatocytes and primary trout hepatocytes. The bioavailability of silver particles in *C. carpio* was investigated by sampling the liver, gill, kidney, brain, blood, gall bladder and intestine. Clear differences in toxicity



between silver and cerium dioxide and between nano-Ag and bulk Ag were observed both *in vitro* and *in vivo*. In *C. Carpio* nano-size silver was seen to be easily taken up and incorporated into tissue (liver, gills, gall bladder and intestine) with ingestion being the most likely main route of uptake. Toxicity to *D. magna* and cell cultures was shown to depend on material, dose and size.

Iain Reid closed the ecotoxicity session with a summary of his work investigating toxicity of nanoparticle carbon black particles in the Great Pond snail *Lymnaea stagnalis*. Adult snails were exposed in a 21 day chronic toxicity test to 260nm and 14.3nm particles and the endpoints of growth, feeding and reproduction were examined. The results of the study show toxicity following nanoparticle exposure, with increased reproductive output and decreased feeding being observed. An increase in physiological stress and reduction in body condition is also seen. The greatest physiological stress was experienced by the 100 mgL<sup>-1</sup> exposure group which showed the least feeding, highest reproductive output and also impaired movement. However, with research still ongoing, Ian explained that the exact mechanisms underlying these responses are yet to be established.

The third session was opened by Bryony Ross, Researcher at the IOM, and Editor of SAFENANO. Bryony gave an overview SAFENANO from her point of view as Editor, covering the Initiative's original conception, its link to SnIRC, how IOM tackled forming and pulling together SAFENANO as a comprehensive 3-tiered offering, and its main achievements to date. In addition, some of the ideas in development for improving and expanding the initiative were outlined, and a subsequent discussion with attending delegates further expanded and added to this.

Dr Steve Hankin then went on to provide an overview of IOM's work on the environmental, health and safety of nanomaterials. Steve outlined some of the key drivers of UK Nano-EHS activity, and highlighted IOM's role filling the gaps between academia, industry, regulators and consumers. Dr Hankin explained that one key area of IOM's campaign to address the significant uncertainties concerning the risks of nanomaterials lies in reviewing and interpreting existing knowledge. A recent project with this focus was the DEFRA funded 'CELL PEN', which sought to scope the research (existing and required) into the mechanisms of nanoparticle translocation across cell membranes, with a particular emphasis on the respiratory epithelium. In addition, the HARN (a review of high aspect ratio nanoparticles), EMERGNANO (a worldwide merging evidence review) and the upcoming ENRHESproject (Engineered Nanoparticles: Review of Environment, Health & Safety [EC]) were highlighted as key pieces of work conducted by IOM in this area.

A second important contribution to the field by IOM lay in aiding prioritisation of research needs and providing strategic advice to Government. The REFNANO project provided a recent example of this type of work. Its aim was to provide a priority list of candidates for inclusion in a set of reference materials to support the measurement, toxicology and risk assessment of engineered nanomaterials in the UK. Finally, Dr Hankin outlined IOM's work toward developing & implementing good practice for nanotechnology. Examples of recent work included production of a BSI Guide for Safe Handling & Disposal of Engineered Nanomaterials, IOM's ongoing work on the ICON Good Wiki Project, and technical development of 'AssuredNano', the UK's first Nanomaterials Accreditation Scheme.

Dr Lang Tran of the IOM then gave an overview of projects within the European Union's 7th Framework Program (FP7) relevant to nanotechnology EH&S. The program is the EU's primary funding mechanism for collaborative research and development projects in science, technology and engineering. Dr Tran provided an overview of IOM's role in successful FP7 projects to date,

including ENHRES, NANOTEST, NANOMMUNE, NANOIMPACTNET, NANOCORE, ObservatoryNANQ and others. Looking forward from the success of the first round of calls, Lang highlighted the need to continue development of FP7 projects involving core SnIRC members in preparation for the next round, mentioning in particular the importance of calls relevant to the areas of exposure assessment, ERA-NET and traffic pollution.

The final session of the day focused on the FP6 project ' Particle Risk ', from which Helinor Johnston of Napier University presented the findings of her investigation into the consequences of hepatocyte exposure to nanoparticles. With multiple studies reporting NP localisation within the liver following exposure through a number of routes, the research aimed to examine in detail the consequences of hepatocyte exposure to a panel of NPs. Using HepG2 cells, C3A cells and hepatocyte couplets in isolation (selected as couplets possess functional bile canaliculi), the research examined the functional consequences of exposure to a battery of NPs using a number of approaches. These included:

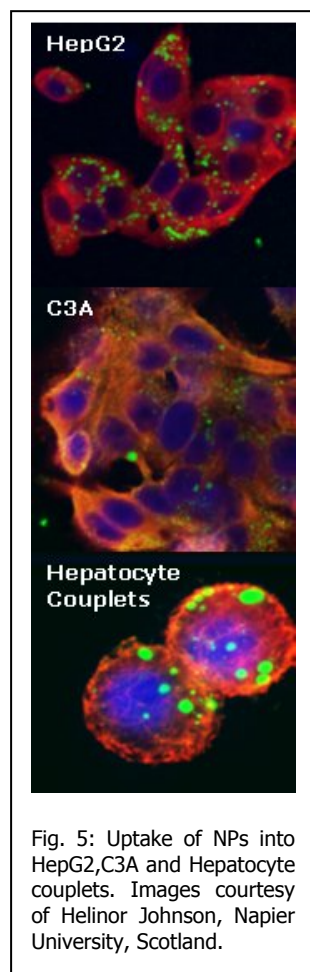


Fig. 5: Uptake of NPs into HepG2, C3A and Hepatocyte couplets. Images courtesy of Helinor Johnson, Napier University, Scotland.

- investigating internalisation of NPs by hepatocytes
- the subsequent intracellular fate of NPs
- the ability of NP exposure to induce oxidative stress using depletion of cellular antioxidant glutathione as an indicator
- assessment of NP exposure on hepatocyte function, including assessment of potential cytotoxicity via assays measuring the release of LDH and MTT activity & impact on CYP activity
- the effect of NPs on bile secretion (using hepatocyte couplets)
- and finally hepatocyte / macrophage interactions, with the cytotoxicity of macrophages examined by measurement of LDH release, and the initiation of an inflammatory response assessed by measuring the release of TNF $\alpha$  using an ELISA.

Although not all of the lab work was quite complete at the time of the meeting, Helinor was able to show clearly that the greatest hepatotoxicity was seen following exposure to quantum dots,

followed by Carbon Nanotubes, with ultrafine Carbon Black affecting bile secretion to an extent.

The meeting closed in style with a visit to Melville Castle, Edinburgh to take part in a 'mini Highland Games' tournament, and a curry and drinks in the city centre. All involved look forward to next years' follow-up meet!



The 'Clanno-Nano' and the 'Haggettes' celebrate their wins; enjoying a well earned drink at the local curry house.

*Bryony Ross & Sarah Harley,  
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