



Nanotoxicology 2010 EDINBURGH

Nanotoxicology 2010: moving from speculation to results

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Nanotoxicology 2010 was held at Napier University's picturesque Craiglockhart campus from the 2nd-4th June. Chaired by Prof Vicki Stone, the conference attracted over 400 delegates from around the world, who were lucky enough to enjoy a week of beautiful sunshine in Scotland's capital city.



The conference commenced from lunchtime on Wednesday 2nd, and provided a programme packed with presentations outlining some of the latest and most exciting findings from

the nano-world, supported by almost 200 poster presentations on a diverse range of areas from characterisation to toxicity and risk assessment. The Colt foundation kindly provided several bursaries to students who demonstrated outstanding work, and the final poster award was presented by Jackie Douglas of the Colt foundation, and Andrew Maynard Director of the Risk Science Center at the University of Michigan School of Public Health, to Micole Massomini of the University of Rome Tor Vergata for her poster "Evaluation of SWCNTs embryo toxicity using in vitro model: The EST."

Co-authored by Julia Varet, Sheona Peters and Bryony Ross, SAFENANO's first feature article of the summer outlines what we considered to be some of the most exciting presentations from what proved to be a hugely successful conference.

Session 1: Setting the scene

Chair: Prof. Vicki Stone & Dr Gary Hutchison, Edinburgh Napier University

The first session of the conference was opened with a keynote presentation by Prof. Günter Oberdörster of the University of Rochester, New York, on 'Nano-particles and Mega-Doses: Relevant for Identifying Adverse Effects?'. Highlighting that both nanotechnology and nanotoxicology appear to be following a typical hype cycle, Prof. Oberdörster began by providing an overview of key studies in the area of nanotoxicology to date and subsequent popular opinion with regards the use of nanoparticles in consumer products. Prof. Oberdörster argued that most of these studies, although important for providing an initial indication of the intrinsic properties of nanomaterials, are of limited value to a risk assessment as they have been performed at unrealistic exposure doses. Moreover, such studies have led to the wide-spread perception that all nanomaterials pose a significant risk to human health. In order to address some of these issues and inspire realistic and reproducible studies, Prof.



Oberdörster provided an outline of some of the key concepts of nanotoxicology, including important considerations in correlating *in vitro* and *in vivo* results (such as dosimetry and biokinetics) and for the design and interpretation of toxicity assays (including key physico-chemical characteristics, influence of the dispersant and analysis of dose-response relationships).

The second presentation of the session came from Georgios Katalagarianakis of the European Commission, who discussed the development of and future direction for the European Commission's Strategy for Nanotechnology Human and Environmental Safety Research. Building on the knowledge and capabilities developed throughout Framework 6 and the first 2 rounds of Framework 7, Dr Katalagarianakis outlined the EC's focuses for the third round of FP7 funding as being threefold:

1. to strengthen the social dimension of nanotechnology EHS research,
2. to ensure high levels of consumer, worker and environmental protection, and
3. to stimulate innovation and support sustainable growth.

On this basis, the third round of EC FP7 funding (2nd half 2010) will be much more Risk Assessment and Life-Cycle Analysis focused, whilst those calls for 2011 will be nanosafety innovation driven. For further information visit the [FP7 website](#) or the [NanoSafety Cluster website](#).

The final presentation of the first session was a valedictory lecture from Professor Peter Gehr of the University of Bern, Switzerland. Spanning thirty years of work in the field of particle-lung interactions, his presentation spanned a multitude of areas from his first Nature publication in 1983 on Fe₃O₄ NPs as probes for intracellular motility, through his career development examining the concept of the airway tree as a particle filter and onto his most recent projects with colleagues both at the University of Bern, and from Harvard School of Public Health, EMPA and the Helmholtz Institute. This varied work includes examination of the toxic effects of break wear particles, the pro-inflammatory effects of silver nanoparticles, triple cell co-culture investigations into the uptake kinetics of CeO₂ NPs, modulation of immune response by biomedical NPs, and examination of the link between the biomechanical effects of NPs on smooth muscle cells and asthmatic conditions. A scientist whose contributions to the respiratory science and nanotechnology fields have been considerable, Prof Gehr will continue to contribute guidance and advice after his retirement through various consultancy positions. Follow this link to [read Peter Gehr's full valedictory abstract](#).

Session 2: Metals

Chair: Jukka Ahtainen (Finnish Environment Agency, Finland)

The second session was opened with a keynote presentation on 'Characterization of Inorganic Nanoparticles for Environmental Fate and Effects' by Dr. James Ranville from [Colorado School of Mines](#), USA. Dr. Ranville began by highlighting the recent growth in consumer products listed as containing nanomaterials and the subsequent increased potential for release of metal-containing nanoparticles (such as silver, zinc oxide and titanium dioxide) into the environment. Although environmental exposure will undoubtedly occur, the nature and extent of the potential ecotoxicological consequences is, as yet, uncertain. In order to advance our knowledge in this area, Dr. Ranville emphasised the importance of developing robust detection, characterisation and quantitation methods to enable the direct measurement of organism exposure and overcome challenges such as background concentrations of nanoparticles in the environment. He then provided an overview of some of the current characterisation techniques available for ecotoxicology and transformation studies, such as [dynamic light scattering \(DLS\)](#) and [UV/Visible spectroscopy](#). In addition, Dr. Ranville highlighted some up and coming methods for using inductively coupled plasma-mass spectroscopy for detecting trace amounts of engineered nanoparticles, including field flow



fractionation (FFF-ICP-MS) and hydrodynamic chromatography (HDC-ICP-MS), relatively new techniques for size separation with subsequent elemental analysis. However, Dr. Ranville stressed the importance of selecting a method that is suitable and appropriate for the system under consideration, using a number of examples to illustrate this point.

Also during session 2, Dr Markus Rehberg of the Ludwig-Maximilians-Universität, Germany presented his work on how 'Quantum dots modulate steps of leukocyte recruitment depending on their surface modification'. Numerous nanomaterials are currently developed for biomedical application. However, Dr Rehberg stressed that it has been shown that some nanoparticles can trigger inflammation and their effects on microcirculation functions are largely unknown. The aim of the study undertaken was therefore to understand the effects of different surface-modified quantum dots (carboxyl, amine and Poly Ethylene Glycol (PEG) surface modified quantum dots) on the recruitment of leukocytes throughout the key stages of: rolling, firm adhesion to the endothelial cells and finally transmigration into the tissues. For this purpose an *in vivo* model which used intra-arterial injection and analysis of the mouse cremaster muscle was chosen. The results indicate that only carboxyl-quantum dots stimulate leukocyte adhesion and transmigration, as observed using *in vivo* microscopy. Moreover, the study indicates that only carboxyl-QD are rapidly taken up by perivascular cells, and further analysis indicates that these perivascular cells are indeed macrophages. These results are in accordance with *in vitro* results showing preferential uptake of carboxyl-quantum dots by Raw 264.7 cells (Mouse leukaemic monocyte macrophage cell line). Finally, Dr Rehberg showed that the carboxyl-quantum dot induced leukocyte recruitment depends on ICAM-1 (Inter-Cellular Adhesion Molecule 1) and can also be attenuated by inhibition of mast cell degranulation. He suggested that the surface modification of the nanomaterial is crucial for its effect *in vivo* and therefore is an important parameter to take into account for the development of biomedical nanoparticles and their functions.

Session 3: High aspect ratio nanoparticles (HARN)

Session Chair: Andrew Maynard (University of Michigan, USA)

This session was opened with a keynote presentation from Ken Donaldson, University of Edinburgh, UK, on 'Asbestos-like effects of HARN in the pleural cavity: approaching an understanding'. Prof. Donaldson began his presentation by discussing fibre length and its link to peritoneal mesothelioma in the rat, one of the earliest contributions to the fibre pathogenicity paradigm. The fibre pathogenicity paradigm is the most robust structure activity relationship in particle toxicology, and it emphasises that the geometry of a fibre as well as its bio-persistence as the main characteristics driving toxicity. Prof Donaldson explained that in an *in vivo* model using the peritoneal cavity of mice, only long carbon nanotubes are found to be inflammatory, this persistent inflammation being a consequence of the failure of macrophages to digest them, called 'frustrated phagocytosis'. Prof Donaldson revisited data and hypothesis from the eighties, suggesting that particle clearance from the peritoneal cavity occurs through parietal stomata of 10µm pore size, draining particles from the cavity into lymphatic system. However, it is postulated that because of their geometry long fibre amosite asbestos and HARN injected into the peritoneal cavity are unable to go through the stomata and are retained in the cavity leading to sustained inflammation. Moreover, there is numerous evidence suggesting that a proportion of all particles deposited in the lung go to the pleural space and are drained via stomata, in the parietal pleura. Then, Prof Donaldson presented *in vivo* data with particles injected into the pleural cavity, showing that injected short carbon nanotubes are rapidly cleared from the pleural space to lymph nodes while long carbon nanotubes induced localised lesions on the parietal pleura. To conclude, Prof Donaldson stressed that the important question for fibre toxicology of the mesothelium is not if the particles reach the pleura after inhalation but if the particles are retained in the pleural space, which is a function of fibre length.



During session 3, a study on 'Biodegradation of carbon nanotubes in the environment and in vivo', conducted by Dr Alexander Star, University of Pittsburgh, USA, was presented. Carbon nanotubes are nanomaterials with numerous interesting properties and therefore their applications and use in numerous fields are on the rise. However, concerns have been raised about their potential toxicity in vivo and few data are available about their persistence in vivo as well as in the larger environment. In this presentation, Dr Star outlined research conducted within his group which shows that single wall carbon nanotubes can be catalytically degraded over time when incubated in presence of the horseradish peroxidase enzyme and hydrogen peroxide. However, while carboxylated single wall carbon nanotubes are catalytically degraded, the group found that pristine ones are not. Following investigation of the mechanism underlying this, it was demonstrated that although the horseradish peroxidase binds the pristine nanotube, its active site cannot degrade the nanotube. It has also been shown that a complete degradation leads to the formation of carbon dioxide, while incomplete degradation can generate various oxidised hydrocarbons. The authors then studied the potential of myeloperoxidase, a peroxidase enzyme expressed mainly in neutrophils and at a lower level in macrophages. It was found that myeloperoxidase catalyses the degradation of carboxylated nanotubes and that these digested nanotubes do not induce inflammation in vivo. Dr Star also stressed the importance of studying the material specific biopersistence of carbon nanotubes in their environmental application. In conclusion, he stated that SWCNTs have limited persistence in the environment, and some physiological mechanisms may exist for their biodegradation - both positive findings in a field which is so dominated by news of the potential hazard CNTs carry.

This session was closed with a presentation from Jürgen Pauluhn, Bayer Schering Pharmaceuticals, Germany, on 'Multi-walled carbon nanotubes (Baytubes®): Risk Characterization and Derivation of Occupational Exposure Limit'. Pauluhn began by outlining the critical parameters for determining a no-observed-adverse-effect-level (NOAEL) in rats (such as alveolar deposition, exposure concentrations and duration etc.) and the Human Equivalent Dose (HED) concept for estimating a NOAEL in humans. Pauluhn then provided an overview of 13-week single and repeated exposure inhalation studies in rat that aimed to assess translocation and kinetics following exposure to multi-walled carbon nanotubes (MWCNT). Key findings of the studies were that lung toxicity appears to be dependent on agglomerate properties rather than on primary particle properties, and that extra-pulmonary toxicity and translocation did not occur. Based on the NOAEL for the 13-week sub-chronic inhalation study in rats, a NOAEL for humans was calculated with interspecies adjustments made to account for differences in alveolar deposition, ventilation and time-dependent particle accumulation. Pauluhn also highlighted the importance of taking workplace specific characteristics such as agglomerate morphology and density into consideration when extrapolating to humans in the occupation setting. Pauluhn concluded that an occupational exposure limit (OEL) of 0.05 mg Baytubes®/m³ (8 hr time weighted average) is supported by both empirical data and volume displacement hypothesis and considered to be protective enough to prevent occupational lung injury.

Session 4: Metal Oxides

Session Chair Wolfgang Kreyling (Helmholtz Zentrum München, Germany)

This session was opened with a keynote presentation from Alison Elder, University of Rochester, USA, entitled 'Metal Oxide Nanoparticles: Lessons from the Past and Questions for the Future'. Nanoparticles possess specific physicochemical properties that make them distinctive from their bulk counterparts. These specific properties related to their nanoscale are interesting for many applications and as a result marketed uses of nanoparticles are increasing. However, these same properties raise also concerns about the interaction of the particles with their environment and potential health and environmental impacts. Assistant Prof. Elder then talked about the numerous nanoparticle-specific



challenges related to risk assessment. For example, physico-chemical characterisation of particles in biological systems is very specific, while metallic nanoparticles dissolved into lung lining fluid, manganese oxide ones showed no sign of dissolution. Such physico-chemical data is very important in understanding the potential effects of nanoparticles upon their target organs at a relevant target organ dose. Asst. Prof. Elder also talked about the need for in vitro assays predicting in vivo toxicity. She demonstrated that by using cellular assays which examine oxidative stress in conjunction with nanoparticle surface area as the relevant metric there was a good correlation with in vivo inflammatory responses. She also stressed that more studies were needed in order to find out if inhaled nanoparticles could induce neurodegeneration, either directly via particle translocation, or indirectly via soluble mediators. Finally, she stressed the need to fill in knowledge gaps in regards to exposure data.

This session was closed with a presentation on 'Nanomaterial Exposure Measurements and Monitoring for Risk Assessment' by Thomas Kuhlbusch from the Institute of Energy and Environmental Technology (IUTA) e.V., Germany. Highlighting that the determination, characterisation, quantification and monitoring of exposure to engineered nanomaterials is important for enabling a risk assessment, Kuhlbusch began by outlining the properties of nanoparticles which of key importance to be characterised: morphology, surface properties, state of agglomeration and stability, and reactive oxygen species (ROS) activity potential. He then went on to overview a number of recent research studies in relation to their use for risk assessment, where exposure measurements were made in the pelletizing and mixing areas of nanomaterial production and processing plants. Kuhlbusch reported that in most cases, the released particles were over 300 nm in diameter. In only a few cases were significant releases of engineered nano-objects of less than 100 nm in diameter measured. Noting that to date mostly stationary measurements have been conducted to derive information on potential exposure, Kuhlbusch concluded his presentation with an overview of some recent advances in the development of personal sampling techniques to allow quantification of personal exposure to nanoparticles.

Session 5: Fate

Session Chair: Ken Donaldson (University of Edinburgh, UK)

The first presentation of the session was given by Peter Wick, of EMPA in Switzerland. His talk, entitled 'Accumulation and translocation of nanomaterials across the human placenta' focussed on the research beginning in the area of reproductive nanotoxicology. Dr Wick began by explaining that background knowledge from epidemiological prospective cohort studies into ultrafine particles in air pollution and early childhood had shown a relationship between raised pollution levels and a decrease in lung function and capacity in neonates. In order to investigate whether nanoparticles may have a similar effect, Dr Wick's group had undertaken a series of studies to investigate the potential for translocation of nanoparticles from mother to child through the placenta, and examine any potential adverse health effects these may cause. Using a re-circulating perfused placental model, which utilises samples of maternal and foetal placental taken shortly post-partum, the group had tested a range of fluorescein beads from 50-500nm in diameter for translocation and viability or functionality changes as a result within the foetal placental tissue. The group found there to be accumulation of beads within placental tissue at diameters less than 240nm, and a clear size-dependent decrease in translocation as NPs increased from 80-240nm in diameter. Within the tissue samples examined by TEM ([glossary link](#)) at completion of the perfusion, fluorescein beads were identified within the brush border, syncytiotrophoblast, stroma and cytotrophoblast (CT) close to the foetal capillary. However, the functionality and viability of the placental tissues were not altered. To conclude, Dr Wick acknowledged that much work is required to develop our understanding of the behaviour of nanoparticles during gestation and post-partum, and that their model showed potential to be a powerful tool to enable toxicological and pharmacological investigation of nanoparticles' behaviour within the placental environment.



This session was closed with a presentation from Dr Anna Shvedova of NIOSH. Her presentation, 'Toxicity of carbon nanotubes to the lung: from mechanisms to regulatory consequences' covered a range of research conducted within NIOSH in the recent years using both aspiration and inhalation exposure at occupational levels, and drew conclusions about how their results contributed to further developing understanding of the relationship between single walled carbon nanotubes (SWCNTs) and pulmonary injury. Shvedova outlined results which linked acute SWCNT exposure to increased acute inflammatory mediators IL-1 β , TNF- α , and raised macrophage levels, sub-chronic exposure increased levels of pro-fibrotic TNF- β and total lung collagen, and chronic (1 year) exposure to bronchioalveolar epithelial cell hypertrophy and bronchiolisation with deposition and residence of SWCNT within tissues. In relation to mechanistic aspects of this, Dr Shvedova also outlined work which had been conducted to investigate the induction of K-Ras oncogene pathways by SWCNTs, highlighting that within the studies undertaken SWCNTs had been observed bridging separating mitotic spindles within cells. To conclude, Shvedova linked some of the group's findings into current occupational exposure limits and regulations, commenting on the relevance and sufficiency for nanomaterials.

Session 6: New and Emerging Topics

Session Chair: Harald Krug (EMPA, Switzerland)

The keynote talk for this session was given by Dr Andrew Maynard, Director of the Risk Science Center at the University of Michigan School of Public Health. A scientist known worldwide for his innovative work in the area of science and policy, and engaging oratory style his presentation, "Nanotoxicology? You ain't seen nothing yet! The challenges of developing emerging technologies responsibly" was as entertaining & thought provoking as expected. Referencing the exponential growth in peer-reviewed publications within the area of nanotoxicology-related topics, Dr Maynard highlighted that we have made much progress in our understanding of nanomaterials and their behaviour (although the number of hazard-related papers still far outweighs those considering exposure; fig 1.). However, he also highlighted that major barriers still exist. For example, the proposal by G-MARINE to use colloidal micelles to attempt to clean up oil from the Deepwater oil spill in the Gulf of Mexico, was halted by protests from NGOs who wrongly associated the product with nanomaterials, and claimed their mass release into the environment was unacceptable. From our experience to date, Dr Maynard went on to propose a set of principles for the focussing research into potential risks arising from emerging technologies. These were:

1. Likelihood of emergent risk (based on current knowledge),
2. Plausibility of risk (to humans and the environment), and
3. Likelihood of a significant impact from these (to humans or the environment).

Using these principles, he re-visited some of the key issues raised in relation to nanomaterials, and to provide the audience with a new perspective on the potential focus areas for the materials nanoscientists handle, he highlighted what he saw as key issues in relation to such materials. This included as priorities materials which:

- are likely to cause abrupt scale-specific changes in biological behaviour,
- can access normally inaccessible areas of the organism/environment,
- are self-assembling,
- undergo activation and change their behaviour in different environments,
- exhibit a scalable hazard not captured by conventional risk assessment, or
- interact synergistically with others in unexpected manners.

The second day of the conference was closed with a presentation by Markus Berges from BGIA <http://www.dguv.de/ifa/en/index.jsp> in Germany on 'Regulating Uncertainty –



Performance Based Benchmark Limits for Nanoparticles in view of Current Measurement Results at the Workplace'. Berges began by highlighting that there are currently no health-based occupational exposure limits (OELs) for nano-objects, the exception to this being in-house company OELs based on limited toxicity data. Faced with the emerging hazards for nanomaterials whose risk probability are still being determined, Berges commented that it would likely be another decade before health-based OELs emerge from the research. A possible solution to this is the development of criteria based on current technical and organisational measures to minimise workplace concentrations. Berges went on to outline a number of requirements which should be met when setting performance-based benchmark levels, including the need to apply the precautionary principle and ensure that the proposed levels are not lower than can be achieved by using conventional engineering controls. Giving consideration to these requirements, Berges outlined proposals for benchmark limit values (BML) of 20,000 particles/cm³ for densities above 6000 kg/m³, and 40,000 particles/cm³ for densities below 6000 kg/m³. However, Berges stressed that these are not health-based limit values and are not applicable to ultra-fine particles. To close his presentation, Berges highlighted the problem of setting a limit value for high-aspect ratio nanoparticles (HARN), given that no standardised sampling or counting methods are available. Although noting that mass concentration- or particle number concentration-based measurements could be used, Berges recommended not using carbon nanotubes adhering to the fibre-paradigm and, where they must be used, employing full enclosure working conditions.

Session 7: Medical Nanoparticles

Session Chair: Bengt Fadeel (Karolinska Institutet, Sweden)

The medical nanoparticles session was opened with a keynote from Martin Philbert, University of Michigan, USA, entitled 'Integrated approach to the early development of nanomaterials for the detection and treatment of brain tumours'. Nanoparticles are promising agents to be used in medicine for diagnosis as well as treatment. Prof. Philbert focused his presentation on the potential application of nanoparticles for brain tumour diagnosis and therapy. Nanoparticles' novel physico-chemical characteristics make them interesting to carry either drugs (for therapy) or contrast agents (for detection). Moreover, additional molecules can be attached onto nanoparticles in order to achieve specific delivery of the drug or contrast agent (for targeting). In addition, nanoparticles have a propensity to stay and accumulate into tumours. Finally, nanoparticles can help to protect their payload and similarly they can reduce systemic toxicity of drugs, by avoiding interactions with the environment before reaching the target site. As an example, Prof. Philbert highlighted the use of nanoparticles to delineate brain tumour for surgery. He described the use of Nanocyan, which is a multifunctional nanoparticle with a core made of biodegradable polyacrylamide, loaded with methylene blue to delineate tumours. These particles are coated with polyethylene glycol to achieve longer plasma circulation and with the F3 peptide for specific targeting of gliosarcoma cells. Nanoparticles can also be loaded with classical drugs, as well as with nucleic acids for gene therapy or photosensitisers for photodynamic therapy. To conclude, Pr Philbert stressed the need for toxicity and pharmacodynamic studies in order to assess the benefit versus risk of these multifunctional nanomedicines, in order to allow their clinical use.

Session 8: Environmental issues

Session Chair: Teresa Fernandes (Edinburgh Napier University, UK)

The session on environmental issues was opened by a keynote from Stig Olsen, Technical University of Denmark, entitled 'Life Cycle analysis + risk assessment'. Dr Olsen began by outlining some of the numerous applications and the potential benefits that nanotechnologies may bring, including for example energy savings, nanomedicine, and reduced use of hazardous chemicals. However, the exponential development and use of nanomaterials also raise concerns about their safety in terms of human health and environmental impact. He



explained that the balance in terms of benefits and risks need to be analysed for every new product application. Risk assessment and life cycle analysis are two methods used in environmental management; while risk assessment is an absolute analysis, life cycle assessment is a relative assessment. Dr Oslon explained that the life cycle of a product includes the extraction of its raw components, production, use and disposal. He highlighted that refinement of the models used for risk assessment and for life cycle assessment are currently subject to similar difficulties because of the large amount of unknown, such as fate, transport, exposure, intake, dose-response relationship, important physico-chemical parameters and other relevant metrics related to nanomaterials. To conclude, Dr Olsen suggested that the evaluation of the impacts of nanomaterials needed to be improved and raised many questions about methodology and strategy used for environmental assessment of nanotechnologies.

But it wasn't just all work...

Aside from a packed conference programme, Nanotoxicology 2010 also hosted two very successful social events for delegates. The first, an official opening drinks ceremony at the Edinburgh City Chambers provided the organising committee and 250 of the delegates with not only the chance to officially celebrate the conference's launch but also with a taste of Edinburgh and Scotland, with speeches from the Lord Provost, a whiskey tasting and entertainment from Robert Burns himself (or at least someone very like him!). The second evening of the conference saw delegates invited to a conference banquet and traditional ceilidh dance at The Hub, a picturesque venue on the city's historic Royal Mile. Both events were maximally attended and enjoyed hugely by those who attended.

...although much effort went into its preparation!

Nanotoxicology 2010 would not have been possible without the hard work and commitment of those on its organising committee. In her conference opening speech, Prof Vicki Stone extended her particular thanks to:

- Dr Gary Hutchinson who took over many of the conference chair duties whilst she was on maternity leave,
- Mrs Lesley Munro without whose administrative and organisational dedication the conference would simply not have happened,
- Miss Fiona Murphy, who in particular helped with the conference packs, bags, transport and ceilidh band,
- Miss Bryony Ross, who organised the conference abstract books, website and banquet venue,
- Prof. Teresa Fernandes, who aided with organisation of the Satellite meetings associated with the event,
- Prof. Ken Donaldson, for his help with final abstract review and selection, and
- Dr David Brown, Dr Julia Varet and other associated Napier University based staff whose help with the conference abstract organisation process was invaluable.

*Bryony Ross, Sheona Peters & Julia Varet
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